Vol. 18 No. 3

PLANT PHYSIOLOGY

JULY, 1943

FOOD RESERVE DEPLETION AND SYNTHESIS IN FIELD BINDWEED, *CONVOLVULUS ARVENSIS* L., AS RELATED TO 7-DAY AND 14-DAY INTERVALS OF CULTIVATION[1]

JOHN C. FRAZIER

Introduction

A study has been made of regenerated bindweed plants which developed following cultivation to a depth of 4 inches. It dealt primarily with the relative depletion of reserve foods by such a cultivation at 7-day and 14-day intervals as determined on plants grown under light-proof covers. Secondarily it sought to obtain information indicating the extent of synthesis of readily available carbohydrates and protein nitrogen in plants in light during the first 8 days following emergence after such a cultivation. The term plant as used in this and in a previous study (**4**) includes the 4-inch rhizome (underground stem) which arises from each root severed at that depth by means of a cultivating instrument and the shoot, or shoots, borne on the same.

A previous paper (**4**), based on the plant material used in this study, showed that on a dry weight basis such bindweed plants, grown in the field under light-proof covers for 8 days after shoot emergence, had shoots that weighed twice as much as the 4 inches of rhizome, the development of which brought about emergence. Since all development of the covered plants must have come from stored food, it appeared logical to conclude that twice the amount of food reserves is required to form the shoots as to form the 4 inches of rhizome. Other workers (**3, 9**) have demonstrated that reserve food depletion in the underground parts of field bindweed grown in full light in the field is continued for at least 8 days after emergence following cultivation. It would appear, therefore, that such plants continue to draw upon the food reserves in the underground parts for this period.

This paper reports findings on the material used in an earlier study (**4**) in terms of the following components: Reducing sugars, total sugars, the starch-dextrin fraction, the computed readily available carbohydrate fraction, and the total nitrogen, protein nitrogen, and the computed "true" protein fractions.

[1] Contribution no. 437, Department of Botany, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Material and methods

The dates on which the material was secured and its previous cultivation treatment are given in table I. Three areas within 8 miles of Manhattan having a loam soil type were used. Area A was a heavy loam, B, light, and C, intermediate in this respect. The precipitation data were given in a previous paper (**4**). The method of securing this material is repeated here. Typical bindweed shoots growing normally in the field were selected as they emerged 6 days after cultivation (to a depth of 4 inches) and immediately covered with an earthen jar of 1- to 2-gallon capacity. The same number

TABLE I

SUMMARY OF FIELD DATA ON REGENERATED PLANTS OF *Convolvulus arvensis*. MANHATTAN, KANSAS, 1940–1941

AREA	DATE	NUMBER OF CULTIVATIONS		NUMBER OF PLANTS	OVEN-DRY WEIGHT OF PLANTS					
					COVERED			EXPOSED		
		1939	1940		4-IN. SECTION OF RHIZOME	SHOOTS	RATIO	4-IN. SECTION OF RHIZOME	SHOOTS	RATIO
	1940				*gm.*	*gm.*		*gm.*	*gm.*	
A	May 3–11	None	None	50	1.133	3.511		0.945	7.142	
B	Aug. 12–20	12	6	40	0.816	1.062		0.784	2.330	
A	Aug. 20–28	None	7	35	0.646	1.407		0.638	3.966	
B	Aug. 30–Sept. 7	12	7	50	0.590	1.308		0.588	2.151	
A	Oct. 5–14	None	10	35	0.301	0.575		0.324	1.216	
B	Oct. 28–Nov. 5	12	10	35	0.414	0.580		0.417	0.750	
				245	3.900	8.443	2.16	3.696	17.555	4.75
	1941	1940	1941							
C	April 14–22	6*	0	35	0.885	1.656		0.923	3.526	
C	June 27–July 5	6*	5	45	0.741	2.496		1.065	7.407	
C	July 11–19	6*	6	65	1.409	4.424		2.285	15.433	
				145	3.035	8.580	2.83	4.273	26.366	6.17

* July 24 to Nov. 4, 1940.

of similar shoots was selected and marked at the same time but was not covered. Eight days later both sets were harvested by severing the shoots at the ground and excavating the 4 inches of rhizome, the development of which had preceded emergence. Thus 4 inches of rhizome (underground part of the stem) and all the leafy shoot (aboveground stem with its leaves) were obtained. The shoots and 4-inch lengths of rhizomes of 245 covered plants and an equal number of plants exposed to sunlight were collected in 1940, and 145 from each situation in 1941.

All plant material secured was killed by holding in a forced circulation electric oven at 105° C. for 20 minutes, following which it was dried 14 hours at 70° C. It was coarsely ground, reduced to 60-mesh size by means of a mortar and pestle and stored in sealed glass containers until analyzed. At

TABLE II

SUMMARY OF ANALYTICAL DATA (ON DRY WEIGHT BASIS) SHOWING THE CONSTITUENT RESERVES IN REGENERATED PLANTS OF *Convolvulus arvensis*. MANHATTAN, KANSAS, 1940–1941

MATERIAL ANALYZED	NUMBER OF PLANTS	OVEN-DRY WT. OF SHOOT OR 4-IN. SECTION OF RHIZOME	REDUCING SUGAR	TOTAL SUGARS	STARCH-DEXTRIN FRACTION	READILY AVAILABLE CARBOHYDRATE FRACTION	READILY AVAILABLE CARBOHYDRATE FRACTION	TOTAL NITROGEN	TOTAL NITROGEN	PROTEIN NITROGEN	PROTEIN NITROGEN	"TRUE" PROTEIN (PROTEIN N × 6.25)
		gm.	%	%	%	%	*mg.*	%	*mg.*	%	*mg.*	*mg.*
1940												
Covered plants	245											
Rhizomes		3.898	1.89	6.06	4.75	10.81	421.4	3.71	144.6	2.95	115.0	718.8
Shoots		8.444	0.82	2.30	3.98	6.28	530.3	5.01	423.0	4.17	352.1	2200.6
Ratio		2.17					1.26				3.06	
Exposed plants	245											
Rhizomes		3.696	2.30	5.42	4.28	9.70	358.5	3.64	134.5	2.89	106.8	667.5
Shoots		17.554	0.64	3.54	6.31	9.85	1729.1	4.88	856.6	3.97	696.9	4355.6
Ratio		4.75					4.82				6.53	
1941												
Covered plants	145											
Rhizomes		3.034	1.80	6.01	4.44	10.45	317.1	3.79	115.0	3.01	91.3	570.6
Shoots		8.577	1.07	2.64	3.88	6.52	559.2	5.09	436.6	4.19	359.4	2246.3
Ratio		2.83					1.76				3.94	
Exposed plants	145											
Rhizomes		4.273	1.80	6.09	4.41	10.50	448.7	3.58	153.0	2.84	121.4	758.8
Shoots		26.365	0.33	3.32	6.44	9.76	2573.2	4.71	1241.8	3.90	1028.2	6426.3
Ratio		6.17					5.73				8.47	

this time it was redried at 105° C., and 2-gram samples for the carbohydrate studies and 0.5-gram samples for the nitrogen studies were weighed out.

The carbohydrate analyses were made essentially as reported by FRAZIER (**5**) except that a 2-gram sample was used and the starch-dextrin fraction was hydrolyzed for an hour at 120° C. in an autoclave. The total nitrogen and protein nitrogen analyses were made as reported by MILLER (**8**). These did not include inorganic nitrogen, since the diphenylamine test showed this to be unnecessary. The "true" protein component was obtained by multiplying the protein nitrogen component by the factor 6.25.

Results

The analytical results are given in table II. These are reported as percentages (on a dry weight basis), or milligrams, or both, of the reducing

TABLE III

READILY AVAILABLE CARBOHYDRATES AND PROTEIN NITROGEN THAT WOULD BE WITHDRAWN FROM THE UNDERGROUND PARTS OF COVERED BINDWEED PLANTS WITH 1 CULTIVATION AT THE END OF 14 DAYS COMPARED WITH 2 CULTIVATIONS AT 7-DAY INTERVALS. MANHATTAN, KANSAS, 1940–1941

CULTURE	1940 245 PLANTS		1941 145 PLANTS	
	READILY AVAILABLE CARBOHYDRATES	PROTEIN NITROGEN	READILY AVAILABLE CARBOHYDRATES	PROTEIN NITROGEN
2 cultivations, *i.e.*, two sets of rhizomes destroyed*	843 mg.	230 mg.	634 mg.	183 mg.
1 cultivation, *i.e.*, one set of rhizomes + 1 set of shoots destroyed	952 mg.	467 mg.	876 mg.	451 mg.
Ratio	1.13	2.03	1.38	2.46
Excess of withdrawal in favor of 1 cultivation	13%	103%	38%	146%

* Cultivation every 7th day permitted no shoot development.

sugars, total sugars, starch-dextrins, readily available carbohydrates, total nitrogen, protein nitrogen and "true" protein in the known weight of a known number of rhizomes and shoots grown under cover and an equal number grown in full light.

In 1940 the shoots of the covered plants contained 1.26 times as much readily available carbohydrates and 3.06 times as much protein nitrogen as the rhizomes of those plants. In 1941 the corresponding ratios were 1.76 and 3.94. For the exposed plants these ratios were 4.82 and 6.53 in 1940 and 5.73 and 8.47 in 1941, respectively.

The relative depletion of food reserves (carbohydrates and nitrogen), supplied by the underground parts of the plant, by cultivation every 7th day as compared with cultivation every 14th day, was calculated for the covered plants. These calculations were made by adding the weight of the readily available carbohydrates or the protein nitrogen found in the rhizome

to the weight of that component found in the shoot (since both would be destroyed by cultivation on the 14th day) and comparing the sum with twice the weight of that found in the rhizome, as two sets of rhizomes, but no shoots, would be destroyed by cultivating at 7-day intervals. The data obtained by these calculations are given in table III. The depletion of readily available carbohydrates was 13 per cent. greater in 1940, and 38 per cent. greater in 1941; and for protein nitrogen the depletion was 103 per cent. greater in 1940, and 146 per cent. greater in 1941 when the one culti-

TABLE IV

COMPARISON OF READILY AVAILABLE CARBOHYDRATES, PROTEIN NITROGEN, AND "TRUE" PROTEIN IN THE RHIZOMES AND SHOOTS OF PLANTS COVERED AND IN LIGHT. MANHATTAN, KANSAS, 1940–1941

MATERIAL ANALYZED	1940 245 PLANTS			1941 145 PLANTS		
	READILY AVAILABLE CARBOHYDRATES	PROTEIN NITROGEN	"TRUE" PROTEIN	READILY AVAILABLE CARBOHYDRATES	PROTEIN NITROGEN	"TRUE" PROTEIN
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Rhizomes						
Exposed plants	359	107	668	449	121	759
Covered plants	421	115	719	317	91	571
Difference	−62	−8	−51	132	30	188
Difference per plant	− 0.25	−0.03	− 0.21	0.91	0.21	1.30
Shoots						
Exposed plants	1729	697	4356	2573	1028	6426
Covered plants	530	352	2201	559	359	2246
Difference	1199	345	2155	2014	669	4180
Difference per plant	4.89	1.41	8.80	13.89	4.61	28.83
Total difference per plant (rhizome plus shoots)	4.64	1.38	8.59	14.80	4.82	30.13

vation was made on the 14th day. In addition such a procedure involved but half the labor.

Based upon the assumption that the rate and extent of reserves supplied by the underground parts were the same for plants in light as for covered plants, calculations were made to compare the average gain in readily available carbohydrates and protein nitrogen for the shoots and rhizomes during the 8-day period following emergence. These data are given in table IV. They show that the average total increase of readily available carbohydrates in the plant (rhizome plus shoots), in light as compared with those covered, for this 8-day period was 4.64 mg. in the 245 plants studied in 1940, and 14.80 mg. in the 145 plants in the 1941 studies. These amounts apparently represent the average synthesis of this fraction per plant during this 8-day interval. This amounts to an average daily gain of 0.6 mg. in the plants studied in 1940, and 1.8 mg. in those studied in 1941; however, this average daily gain is given for comparative purposes only, as probably most of it

accumulated the last 2 or 3 days of this 8-day period, *i.e.*, after the shoot had established its green surfaces.

The total nitrogen accumulation represents inorganic nitrogen which had been utilized in the formation of organic nitrogenous compounds, particularly proteins, plus that which entered the plant parts studied because of the inorganic nitrogen in those parts being so utilized.

The data of table IV show that the average total increase of protein nitrogen in the rhizome plus shoot, in light, for this 8-day period was 1.38 mg. in the 245 plants used in 1940, and 4.82 mg. in the 145 plants studied in 1941. The calculated average daily increase per plant was 0.17 mg. in 1940, and 0.6 mg. in 1941.

Another possible explanation of the greater amount of reserves in plants growing in light is that part or all was obtained from root reserves. Additional studies are contemplated in which a 4- to 6-inch section of the roots which bear the rhizomes will be obtained in addition to the rhizomes and shoots of plants growing in light and under light-proof covers. If no appreciable difference is found in the reserve content of these roots, definite evidence will have been obtained that the difference in reserves in rhizomes plus shoots growing in light and under light-proof covers is the result of photosynthesis occurring in those in light.

Discussion

Successful control of noxious perennial weeds has long been based upon the suppression of shoot growth in order that the underground parts be depleted of their food reserves. In recent years it has been recognized that shoots may emerge and grow for several days before returning food reserves to the underground parts (**3, 7, 9, 11**). TIMMONS (**10**) reported that in most cases the decrease in reserves was more rapid for several days after than prior to emergence of the bindweed following cultivation.

The 8-day interval following emergence, used in this study, added to the 6 days commonly required under average growing conditions to bring about emergence, when cultivations are made to a depth of 4 inches, gives the 14-day cultivation interval recommended for bindweed control in Kansas (**6**).

The term "readily available carbohydrates" as commonly understood includes the total sugars and the starch-dextrin fraction. Recent work (**1, 3, 10, 11**) indicates that this composite fraction is the most meaningful one in the carbohydrate economy of the plant. It is generally agreed that all portions of this fraction (freed of gums by clearing) are readily utilized as food reserves by the plant if, as BAKKE *et al.* (**1**) stated, depletion can be taken as a measure of utilization.

The differences noted in the food reserve relations between the two growing seasons is believed due in part to the more vigorous shoot growth found in the patch studied in 1941 plus the fact that two of the three studies made that year were at the time of marked growth, while only one of the six studies made in 1940 was during the more vigorous early spring growth.

Possibly the rhizomes secured in this experiment were different in their food reserve relations than had they been taken at the time of their emergence. To ascertain if such is the case, 25 rhizomes were taken at each of the 3 periods (table I) of sampling in 1941 or a total of 75. These weighed 1.209 gm. of which 144.96 mg., or 11.99 per cent., consisted of readily available carbohydrates. This is an average of 1.93 mg. per rhizome. This does not seem significantly different from the 1.89 mg. average weight found in the 390 rhizomes (1940–1941) grown under cover which is the group with which this comparison should be made. No comparable study was made for the nitrogenous fractions.

The percentages of the carbohydrate fractions may appear low but the bindweed being studied was subjected to cultivation at 14-day intervals. They are in general agreement with findings on bindweed subjected to such treatment in Colorado (**2**). The specific cultivation treatment given the plants reported in this study is to be found in table I.

Studies on the nitrogenous fractions have been reported by other workers (**1, 3, 7, 10**). There is general agreement that the rôle played by these fractions is not well understood at present. BAKKE *et al.* (**1**) reported that both colloidal and noncolloidal nitrogen were reduced in plants grown on fallowed land. BARR (**3**), however, found that the soluble-nitrogen content remained about the same in either cultivated or undisturbed bindweed roots, while cultivation reduced the colloidal- or protein-nitrogen content, although frequency of cultivation appeared to have no material effect. These investigators (**1, 3**) used a different reagent for fractionation, hence no direct comparison can be made with the work reported herein. BARR (**3**) reasoned that such a shift with the soluble nitrogen materials going into the developing shoot would account for the rapid reduction he observed in protein nitrogen after cultivation, a reduction which continued for at least 10 to 15 days after cultivation.

The present study indicates that there is a rapid accumulation of nitrogenous materials in the rhizomes and shoots once they start developing and that a large proportion, approximately 80 per cent., is present as protein nitrogen. Because of the method of calculation the trends of "true" proteins paralleled those obtained for protein nitrogen.

Summary

1. Regenerated bindweed plants which developed following cultivation to a depth of 4 inches were studied. These consisted of the 4-inch section of rhizome which arose from roots severed at that depth and the shoot, or shoots, borne on that rhizome.

2. Comparable numbers of such regenerated plants were studied after 8 days of shoot development under normal sunlight and under light-proof covers.

3. The material was assembled into four composite samples, *viz.*, rhizomes exposed, shoots exposed, rhizomes covered, and shoots covered, for each year

of the study. These were analyzed for various carbohydrate and nitrogen fractions. The data are reported as percentages (on a dry-weight basis) or milligrams, or both, of the reducing sugars, total sugars, starch dextrins, readily available carbohydrates, total nitrogen, protein nitrogen, and "true" protein.

4. The relative amounts of the two fractions (the readily available carbohydrates and the protein nitrogen) in the two parts, rhizomes and shoots of plants grown under each light condition, covered and exposed, were determined for the two growing seasons.

5. The relative depletion of food reserves, supplied by the underground parts of the plant, by cultivation every 7th day as compared with cultivation every 14th day, was calculated. From this it appears that cultivation every 14 days would destroy a fifth more of the readily available carbohydrates and more than double the loss of protein nitrogen in the two parts as compared with two cultivations at intervals of 7 days in the same unit of time.

6. Synthesis by the plants growing in light is considered to account for the higher reserves of those plants. Based upon the assumption that the rate and extent of reserves supplied by the underground parts were the same for plants in light as for covered plants, data are presented showing the average gain per plant of readily available carbohydrates and of protein nitrogen for the 8-day period of shoot development following emergence and the average gain of these fractions per plant per day for the two growing seasons.

KANSAS STATE COLLEGE
MANHATTAN, KANSAS

LITERATURE CITED

1. BAKKE, A. L., GAESSLER, W. G., and LOOMIS, W. E. Relation of root reserves to control of European bindweed, *Convolvulus arvensis* L. Iowa Agr. Exp. Sta. Res. Bull. 254. 1939.
2. BARR, C. GUINN. Preliminary studies on the carbohydrates in the roots of bindweed. Jour. Amer. Soc. Agron. **28**: 787–798. 1936.
3. ————————. Organic reserves in the roots of bindweed. Jour. Agr. Res. **60**: 391–413. 1940.
4. FRAZIER, JOHN C. Influence of time of cultivation on the control of field bindweed, *Convolvulus arvensis* L. Trans. Kansas Acad. Sci. **44**: 164–169. 1941.
5. ————————. Amount, distribution and seasonal trend of certain organic reserves in the root system of field bindweed, *Convolvulus arvensis* L. Plant Physiol. **18**: 167–184. 1943.
6. The bindweed program of Kansas. Report of the State Board of Agriculture. Vol. **57**, no. 227. 42 pp. 1938.
7. KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W. Bindweeds and their control. Nebraska Agr. Exp. Sta. Bull. 287. 1934.

8. MILLER, E. C. A physiological study of the winter wheat plant at different stages of its development. Kansas Agr. Exp. Sta. Tech. Bull. 47. 1939.
9. TIMMONS, F. L. Annual report from the bindweed control project. Fort Hays (Kansas) Branch Exp. Sta. 1937. Unpublished. (pp. 43 and 51 cited.)
10. ————. Annual report from the bindweed control project. Fort Hays (Kansas) Branch Exp. Sta. 1939. Unpublished. (p. 42 cited.)
11. ————. Results of bindweed control experiments at the Fort Hays Branch Station, Hays, Kansas, 1935 to 1940. Kansas Agr. Exp. Sta. Bull. 296. 1941.

NUTRIENT DEFICIENCIES IN THE STRAWBERRY LEAF AND FRUIT[1]

R. A. Lineberry and Leland Burkhart

(WITH SEVEN FIGURES)

Introduction

Previous investigational work on the nutrition on the strawberry has been concerned largely with the relationship of soil and fertilizer treatment to growth and yield (**5**). While these studies have increased greatly the knowledge of the fertilizer requirements of the strawberry, they did not describe the symptoms of mineral deficiencies nor show the mineral composition of the plant and fruit. It would appear that foliar symptoms and mineral composition of the plant might be correlated with nutritional needs. In this connection the most notable contribution has been made by Davis, Hill, and Johnson (**4**). They found that foliar symptoms could be correlated with nutritional needs and that certain positive and negative correlations existed between pairs of elements in the ash of the plant. These relationships were established for the Parson Beauty variety grown under outdoor conditions in Canada. Water color illustrations of potassium-, phosphate-, and nitrogen-deficient strawberry leaves (variety not mentioned) have been published by Davis and Hill (**3**). Hoagland and Snyder (**6**) have reported on the effects of potassium, phosphate, chloride, and boron on the growth of the strawberry plant in water cultures in California.

The effect of certain mineral deficiencies on leaf characteristics and associated mineral composition of the foliage and fruit of the strawberry is reported herein. The Klondike and the Blakemore were used as these are the two leading commercial varieties grown in North Carolina.

Materials and methods

In this study, dormant plants of Blakemore and Klondike, with fruit buds well formed, were removed from uniformly fertilized fields to the greenhouse early in January. The roots were washed free from soil, the old leaves were removed, and the plants were set in washed quartz sand in two-gallon glazed earthenware containers which were provided with drainage. Two plants were set in each container and twenty plants of each variety were included in each treatment. The mineral content of dormant field-grown Klondike strawberry plants was determined at the beginning of the experiment. The composition of the respective nutrient solutions employed is shown in table I. These solutions were buffered at pH 5.6 and were applied daily, using the sand-culture technique. Preliminary studies of the

[1] Contribution from the Department of Agronomy, N. C. Agricultural Experiment Station and Div. of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Published with the approval of the Director as Paper no. 150 of the Journal Series.

growth of strawberry plants in sand cultures indicated that the presence of chlorides in the nutrient solutions was objectionable except at very low concentrations. High concentrations of sulphate ions also proved objectionable. The chloride ion was, therefore, omitted from all nutrient solutions and sulphate concentrations were kept low (table I).

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS*

SERIES	PARTIAL VOLUME MOLECULAR CONCENTRATION OF SALTS									
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaSO_4$	NaH_2PO_4	$NaNO_3$	Na_2SO_4	K_2SO_4	NH_4NO_3	$Mg(NO_3)_2$
Complete	0.004	0.002	0.002	.....	.....	.....	.....	.....	0.001	.....
Minus-Ca	.....	0.002	0.002	.....	.....	0.008	.....	.....	0.001	.....
Minus-K	0.004	.....	0.002	.....	0.002	.....	.....	.....	0.001	.....
Minus-Mg	0.004	0.002	.....	.....	.....	.....	0.002	.....	0.001	.....
Minus-P	0.004	.....	0.002	.....	.....	.....	.....	0.007	0.001	.....
Minus-S	0.004	0.002	.....	.....	.....	.....	.....	.....	0.001	0.002
Minus-N	.....	0.002	0.002	0.004	.....	.....	.....	.....	.....	.....

* Boron at the rate of 0.5 p.p.m. and manganese, iron, zinc, and copper at the rate of 0.25 p.p.m. were added to all nutrient solutions.

The studies were made during the fruiting stage of development in January and February. Mature leaves showing deficiency symptoms were sampled for chemical analysis and ripe fruits from the plants receiving the various treatments were sampled at the time the leaf deficiency symptoms appeared. The fresh leaves and fruit were extracted and analyzed by the procedure described by BURKHART and PAGE (1).

Results

NUTRIENTS IN FOLIAGE AND FRUIT

At the beginning of the experiment, samples of dormant field-grown Klondike plants were taken for the determination of the soluble minerals present in the leaf blades, petioles, crowns, and roots (table II). In the dormant state, there was a considerable accumulation of potassium in the roots as compared with that in leaves and crowns. Soluble calcium, mag-

TABLE II

DISTRIBUTION OF SOLUBLE MINERALS IN DORMANT KLONDIKE STRAWBERRY PLANTS, CONSTITUENTS EXPRESSED AS P.P.M. OF THE FRESH TISSUE

	Ca	K	Mg	PO_4	SO_4
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Leaf blades	2325	2750	2625	5000	400
Petioles	550	3750	2000	1500	250
Crowns	200	2250	1000	2000	2500
Roots	1100	4000	1500	1500	2000

nesium, and phosphate were somewhat localized in the leaf blades. Petioles were low in soluble calcium and sulphate and the crowns were relatively very low in soluble calcium.

After growing in sand culture for about two months, until they fruited and deficiency symptoms were apparent, determinations were made of the soluble-nutrient levels in both leaves and fruit of the Blakemore variety, with the results shown in figures 1 and 2. The potassium concentrations of

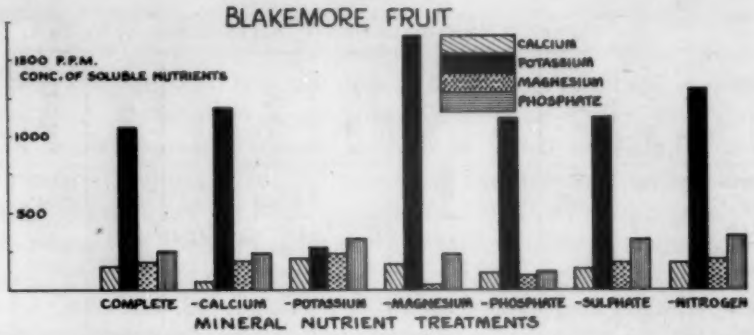


FIG. 1. Soluble nutrients (expressed as parts per million in foliage and fruit) found in Blakemore strawberry plants, grown in nutrient solutions with the indicated deficiencies in certain elements, and in complete nutrient solution.

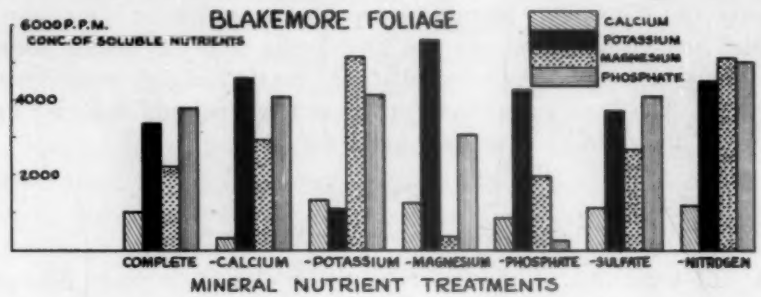


FIG. 2. Soluble nutrients (expressed as parts per million in foliage and fruit) found in Blakemore strawberry plants, grown in nutrient solutions with the indicated deficiencies in certain elements, and in complete nutrient solution.

both leaves and fruit were greatly affected by the ionic nature of the nutrient. Lack of calcium, and especially of magnesium, resulted in a marked increase in potassium in the foliage and the fruit as compared with the effects of the complete nutrient treatment. Plants grown in the potassium-deficient nutrient solution produced foliage containing 1200 p.p.m. of potassium and fruit with 400 p.p.m. of this element, each value being about one-third of that for plants in the complete nutrient series. In the complete nutrient treatment the potassium concentration in relation to the other three soluble minerals was much less in the fruit than in the leaves. There was practically no soluble phosphate in either foliage or fruit at the time phosphorus deficiency symptoms were evident.

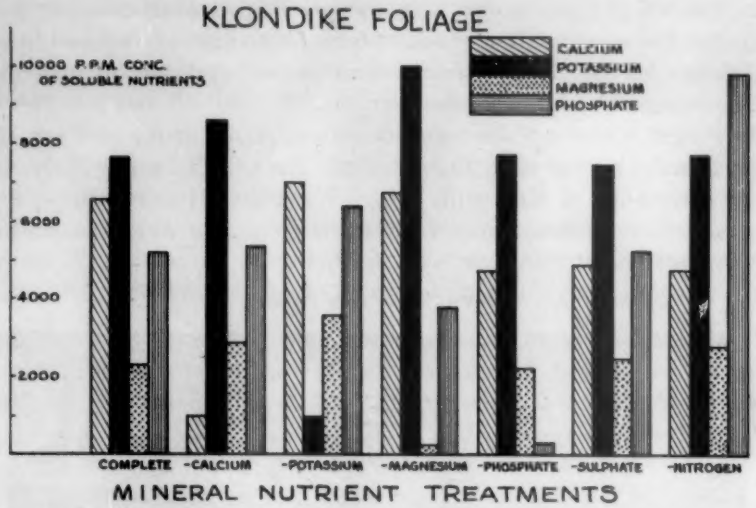


FIG. 3. Soluble nutrients found in Klondike strawberry plants grown in solutions with the indicated deficiencies in certain elements and in complete nutrient solution. Expressed in parts per million of fresh foliage and fruit.

In the Klondike variety (figs. 3 and 4) the concentration of soluble potassium and calcium in both leaves and fruit of plants that received complete nutrient solution were much higher than in the Blakemore variety. The ionic antagonistic effects of potassium and magnesium upon the concentrations of their ions in the foliage and fruit of Klondike were very much the same as in the Blakemore. The relatively high potassium concentration in the fruit of Klondike in relation to other soluble minerals was also evi-

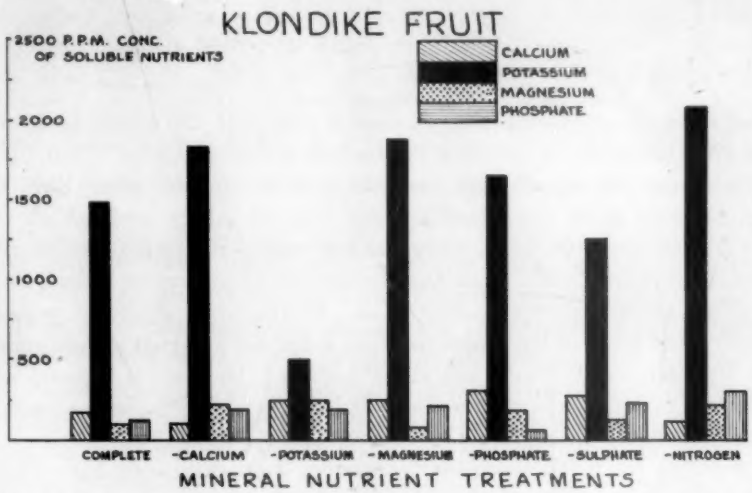


FIG. 4. Soluble nutrients found in Klondike strawberry plants grown in solutions with the indicated deficiencies in certain elements and in complete nutrient solution. Expressed in parts per million of fresh foliage and fruit.

dent. Soluble phosphate also accumulated in potassium-deficient foliage and fruit. The accumulation of soluble phosphate was very marked in nitrogen-deficient leaves. In the fruit, potassium accumulation in high degree was associated with nitrogen deficiency.

DAVIS *et al.* (**4**) also found negative correlations in the leaf ash of the Parson Beauty variety of K_2O with CaO, MgO, P_2O_5, respectively, but a positive correlation of MgO with P_2O_5. WALLACE (**8**) has reported a high nitrogen content of strawberry fruit when potassium was deficient in the nutrient medium.

DEFICIENCY SYMPTOMS

POTASSIUM DEFICIENCY.—A distinct varietal difference was noted in the potassium deficiency symptoms on the leaves. In the Klondike variety, potassium deficiency was characterized by progressive necrosis of the petioles just below the leaflets as shown in figure 5, B and C. Concurrently the mid-

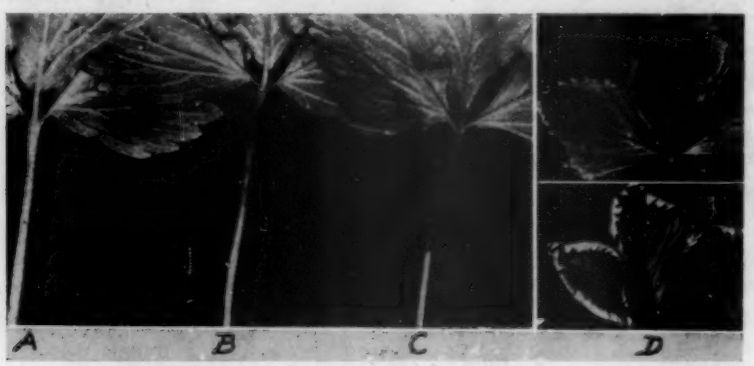


FIG. 5. Potassium deficiency symptoms in strawberry leaves. A, healthy leaf of the Klondike variety; B and C, progressive necrosis in petioles of this variety; D, marginal leaf scorch in the Blakemore variety.

ribs of the leaflets became purple. As a result of the breakdown of the conducting tissue in the petioles the leaflets wilted and soon dried up. In the Blakemore variety, though analysis showed the potassium level to be about the same as in the Klondike, this type of injury was not observed. In the Blakemore there was a marginal necrosis of the leaflets which gradually rolled upward and inward (fig. 5, D) and the younger leaflets became light green to yellow between the veins. In the early stages of potassium deficiency, the fruit of both varieties had a normal external appearance but many ripe Klondike fruits had dead calyces. In more advanced stages of potassium deficiency, the wilting and drying up of the pedicels and peduncles resulted in considerable shriveling of fruits.

DAVIS and HILL (**3**) described potassium deficiency symptoms (variety not mentioned) in the following manner: "In early stages the plants were dark green in color which lasted well into the fruiting season; however, they were smaller and less vigorous than those receiving complete nutrients.

The leaves gradually lost their luster and by the end of the fruiting season began to curl. A bronzing and considerable purpling was observed on the under surface of the leaflets by late summer."

HOAGLAND and SNYDER (**6**) in studying potassium deficiency of the Marshall, Ohmer, and Klondike strawberries did not find marginal scorch prominent in any of these varieties, but reported bronzing and necrosis of the petiole and base of the blade.

CALCIUM DEFICIENCY.—In both the Blakemore and Klondike varieties, symptoms of calcium deficiency first appeared as injury to the roots followed by injury to the leaves. A marked varietal difference in the appearance of

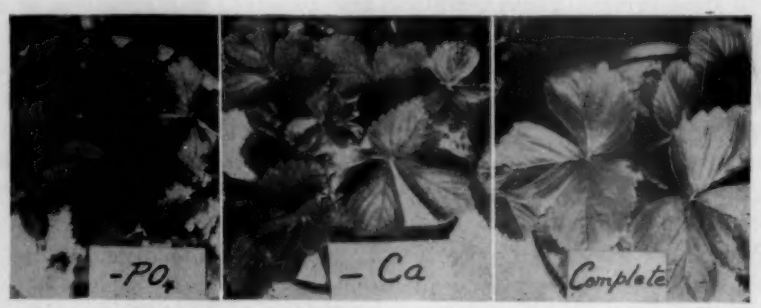


FIG. 6. Symptoms of phosphate and calcium deficiencies in Blakemore strawberry plants.

FIG. 7. Symptoms of phosphate and calcium deficiencies in Klondike strawberry plants.

calcium deficient leaves was evident. Calcium deficient Blakemore plants made much more growth than did the Klondike plants (figs. 6 and 7). Symptoms in the Blakemore foliage, however, were evident as in figure 4. The younger leaves were much deformed and crinkled, and exhibited tip burn. In contrast, the younger Klondike leaves were not crinkled but the older leaves lost their luster and became somewhat mottled. Later these older leaves became flaccid and internal breakdown of tissue was evident, suggesting that calcium had been translocated to some extent from them to the younger leaves. The fruits of calcium deficient plants of both varieties were very much deformed while still green and were still small when ripe.

The calyces of the ripe fruit of the Klondike variety all died, a condition also noted in potassium deficiency.

DAVIS *et al.* (**4**) reported that calcium deficient plants of the Parson Beauty strawberry grew vigorously during the summer and did not develop leaf blotch until early fall, after which leaf growth was restricted.

MAGNESIUM DEFICIENCY.—Early stages of magnesium deficiency in the Klondike foliage were characterized by the downward and inward rolling of the yellowish-green leaf margins. In later stages the upper surface of the leaf blades between the veins became a yellowish-orange color except in the region along the midrib. Brown necrotic areas appeared on the under surface of these leaves. These symptoms also appeared in magnesium deficient Blakemore foliage.

DAVIS *et al.* (**4**) reported that in summer the magnesium deficient series were the most vigorous of his experimental plants. The large leaves were of normal color until late August when characteristic brown patches, mostly confined to the leaf margins, developed. Abscission of these brown patches gave the leaves a ragged appearance. The petioles were always longer than those in the normal series.

PHOSPHATE DEFICIENCY.—Early stages of phosphate deficiency in both varieties were characterized by an intensified blue-green coloration of the foliage. This was accompanied by reddening of the leaf margins. In later stages the entire surface of the older leaf blades became bronzed and purpled due to the red pigments overlying the blue green. The petioles became red and brittle and the midribs and veins on the under surface of leaf blades showed the purple coloration that suggested a physiological nitrogen deficiency. The leaves were not as thick as the leaves of normal plants and the petioles were shorter. In more advanced stages of phosphate deficiency the plants were much dwarfed and the older leaves became brown and dry. DAVIS *et al.* (**4**) observed similar symptoms in the Parson Beauty variety in Canada.

NITROGEN DEFICIENCY.—In early stages of nitrogen deficiency the serrations at the margins of older leaves became red. As nitrogen deficiency progressed, the younger leaves developed more slowly and appeared yellowish green. Later a reddening gradually extended over the entire leaf surface. The petioles, which were shorter than in the plants receiving complete nutrients, became red and brittle. In advanced stages of nitrogen deficiency the older leaves became light yellow with browning and necrosis of localized areas. The ripe fruit was small and the plants developed few feeding roots. In general, these nitrogen deficiency symptoms are in agreement with those described by DAVIS and HILL (**2**) and by DAVIS *et al.* (**4**). For the Aroma strawberry, LONG and MURNEEK (**7**) found that the nitrogen content of leaves decreased rapidly during senescence, with a simultaneous increase in the roots and stems. They further reported that the roots accumulated 30 to 40 per cent. of the total amount of nitrogen found in the plant during the winter.

Differences in symptoms

The mineral deficiency symptoms in the leaves of the Klondike and the Blakemore varieties as grown under the conditions reported herein are summarized in table III. Where there were differences in the symptoms between the young and old leaves such differences are listed. Varietal differences were greatest in the symptoms of calcium and potassium deficiencies. These differences were evident in the young leaves for calcium deficiency,

TABLE III

Nutrient deficiency symptoms in leaves of two varieties of strawberries

Nutrient deficiency	Variety	Symptoms
Calcium	Klondike	Young leaves: dwarfed, with marginal scorch, followed by death of buds. Mature leaves*: loss of luster and turgor, followed by mottling.
	Blakemore	Young leaves: dwarfed and crinkled near tips. Mature leaves: loss of luster and turgor followed by yellowing.
Potassium	Klondike	Mature leaves: progressive purpling of midrib and petiole, followed by necrosis of petiole.
	Blakemore	Mature leaves: margins of leaf blades scorched and rolled upward.
Magnesium	Klondike and Blakemore	Mature leaves: chlorosis of outer portion, followed by downward rolling of leaf margin.
Phosphate	Klondike and Blakemore	Young leaves: dark blue-green, and retarded in growth. Mature leaves: intensified blue-green accompanied by reddening of the leaf margins, the leaf blades later becoming bronzed and purpled and petioles bright red. Midribs and veins on under surface of leaf becoming purpled.
Nitrogen	Klondike and Blakemore	Young leaves: in more advanced stages, light green to yellow; growth restricted. Mature leaves: in early stages, the serrations of older leaves redden. Later the entire leaf surface reddens, the older leaves finally becoming bright yellow accompanied by necrosis and browning of localized areas.

* A mature leaf as used herein refers to fully expanded turgid leaves.

and in the mature leaves for potassium deficiency. No varietal difference in symptoms was observed for magnesium, phosphorus, or nitrogen deficiency. Hoagland and Snyder (6) found Nick Ohmer strawberry to be less susceptible than Marshall to nutrient deficiencies, and Klondike to be the variety least susceptible to boron deficiency.

Discussion

The composition of the leaves of plants given a complete nutrient solution for about 2 months differed from that of dormant plants at the beginning of the experiment chiefly in the greater content of potassium. Analysis of

the leaves of plants in the deficiency tests after symptoms were evident indicates that their calcium, magnesium, and phosphorus content, as well as potassium content, may vary greatly depending on the supply. It would seem, therefore, that foliar analysis as an index to the nutrient condition of the plant might be practical.

In general, the nutrient content of the strawberry fruit followed that of the leaves but was not so high. Characteristic foliage symptoms of nutrient deficiencies were observed. For the most part they correspond to those observed in the strawberry by other investigators but additional symptoms were recorded. Particularly evident were the differences in the calcium and potassium deficiency symptoms for the Klondike variety as compared with the Blakemore. This indicates the need for a study of the symptoms for different varieties in order to make a correct diagnosis of deficiencies.

Summary

1. Foliar symptoms of potassium, calcium, magnesium, phosphate, and nitrogen deficiencies are described for both Blakemore and Klondike strawberry varieties during the fruiting stage of growth.

2. Marked varietal differences in potassium and calcium deficiency symptoms are noted and illustrated.

3. Soluble minerals were determined in the foliage and fruit of plants receiving the various nutrient treatments, and the results obtained are illustrated.

4. In the complete nutrient treatment of each variety the potassium concentration in relation to other soluble minerals in the fruit was high as compared with the same relationship in the leaves.

5. There were only traces of soluble phosphate in the foliage and fruit at the time phosphate deficiency symptoms were evident.

Division of Fruit and Vegetable Crops and Diseases
Bureau of Plant Industry, The U. S. Department of Agriculture
and
The North Carolina Agricultural Experiment Station
Raleigh, North Carolina

LITERATURE CITED

1. Burkhart, Leland, and Page, N. R. Mineral nutrient extraction and distribution in the peanut plant. Jour. Amer. Soc. Agron. **33**: 743–755. 1941.
2. Davis, M. B., and Hill, H. Nutritional studies with Fragaria. Sci. Agr. **8**: 681–691. 1928.
3. ————, and ————. Nitrogen, phosphoric acid, and potash starvation at different stages of growth of Fragaria. Canada Dept. Agr. Pamphlet no. 96. 1928.
4. ————, ————, and Johnson, F. B. A study of the effect of deficient and excess potassium, phosphorous, magnesium, calcium, and sulphur. Sci. Agr. **14**: 411–432. 1934.

5. GARDNER, V. R. Studies in the nutrition of the strawberry. Missouri Agr. Exp. Sta. Res. Bull. 57. 1923.
6. HOAGLAND, D. R., and SNYDER, W. C. Nutrition of strawberry plant under controlled conditions. Proc. Amer. Soc. Hort. Sci. **30**: 288–294. 1933.
7. LONG, J. H., and MURNEEK, A. E. Nitrogen and carbohydrate content of the strawberry plant. Missouri Agr. Exp. Sta. Res. Bull. 252. 1937.
8. WALLACE, T. The nutrition and fertilizing of soft fruits. Imp. Bur. Fruit Production Tech. Communication **6**: 52–87. 1936.

AN INSTALLATION OF LARGE SAND-CULTURE BEDS SURMOUNTED BY INDIVIDUAL AIR-CONDITIONED GREENHOUSES[1]

MOYER D. THOMAS, RUSSEL H. HENDRICKS, JAMES O. IVIE, AND GEO. R. HILL

(WITH TEN FIGURES)

In this series of papers, various phases of the relations of sulphur to plant growth are being considered. Special consideration is given to the rôle of sulphur dioxide. This study was initiated in 1935–1936, when experiments were carried out at Logan, Utah (**8, 9**), in a non-industrial atmosphere sufficiently free from sulphur compounds to permit the study of the effects of low concentrations of sulphur dioxide on alfalfa. In order to continue the studies at this laboratory, it has been necessary to set up special equipment to control the sulphur supply, both to the roots and to the leaves of the vegetation. This has been accomplished by the construction of a group of large automatically-irrigated, sand-culture beds surmounted by individual greenhouses, supplied with washed and filtered air.

The utility of the sand bed is well established in solution-culture work, primarily because the sand provides support for the plants and permits good aeration of the roots, as well as control of soluble materials available to them. Further, it is not difficult to irrigate the plants uniformly. Normal growth of the vegetation and good yields have been repeatedly observed. For example, ARNON and HOAGLAND (**1**) grew tomatoes in the greenhouse in comparable sand, water, and soil cultures. They obtained excellent growth in all three media, but the yield of fruit was somewhat higher in the sand than in the water or soil. In our experience, root respiration measurements and transpiration measurements can be readily carried out in the sand bed. Also, it is not difficult to harvest the roots quantitatively.

In addition to the usual sand-cultures in pots, crocks, or on greenhouse benches, special small and large sand containers, and equipment for irrigating them automatically on the surface, have been described by EATON (**4, 5, 6**) and by CHAPMAN and LIEBIG (**3**). Subirrigated sand and gravel beds have been used by BIEKART and CONNORS (**2**) and by WITHROW and BIEBEL (**11, 12**). Some of these installations were designed for scientific studies in plant nutrition; others for commercial crop production.

The following features were sought for the equipment described in this paper:

1. Sand beds large enough to grow a number of plants to maturity, with root systems of as nearly normal size as possible.

2. Airtight greenhouses surmounting the beds, in which the gaseous environment could be controlled.

[1] This is the third of a series of papers on "The Effect of Prolonged Low Concentrations of Sulphur Dioxide Upon Plants."

3. Containers with insoluble walls that would supply as small an amount of nutrients as possible.

4. Automatic irrigation.

5. Ease of control of concentrations and volumes of the nutrient solutions, and of air with known sulphur dioxide concentrations.

6. Facilities for root respiration and photosynthesis measurements.

Installation

A diagram of one unit of the equipment is given in figure 1, and a section of the general assembly is shown in figure 2. Figure 3 is a photograph of

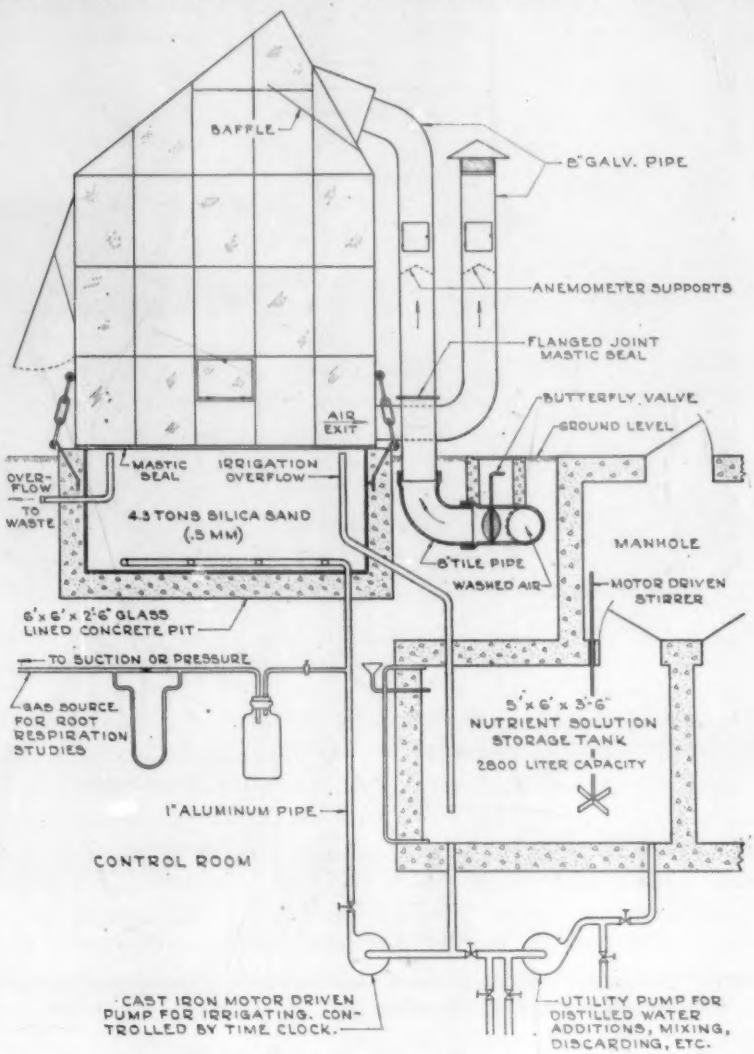


FIG. 1. Diagrammatic elevation of one unit of the sand culture equipment.

the installation, looking southwest, and showing, in addition to the greenhouses, the location of the air-washing unit, the recorder room, and a large concrete pit for storing and treating the sand. Figure 4 shows the greenhouses filled with first crop alfalfa on April 29, 1941.

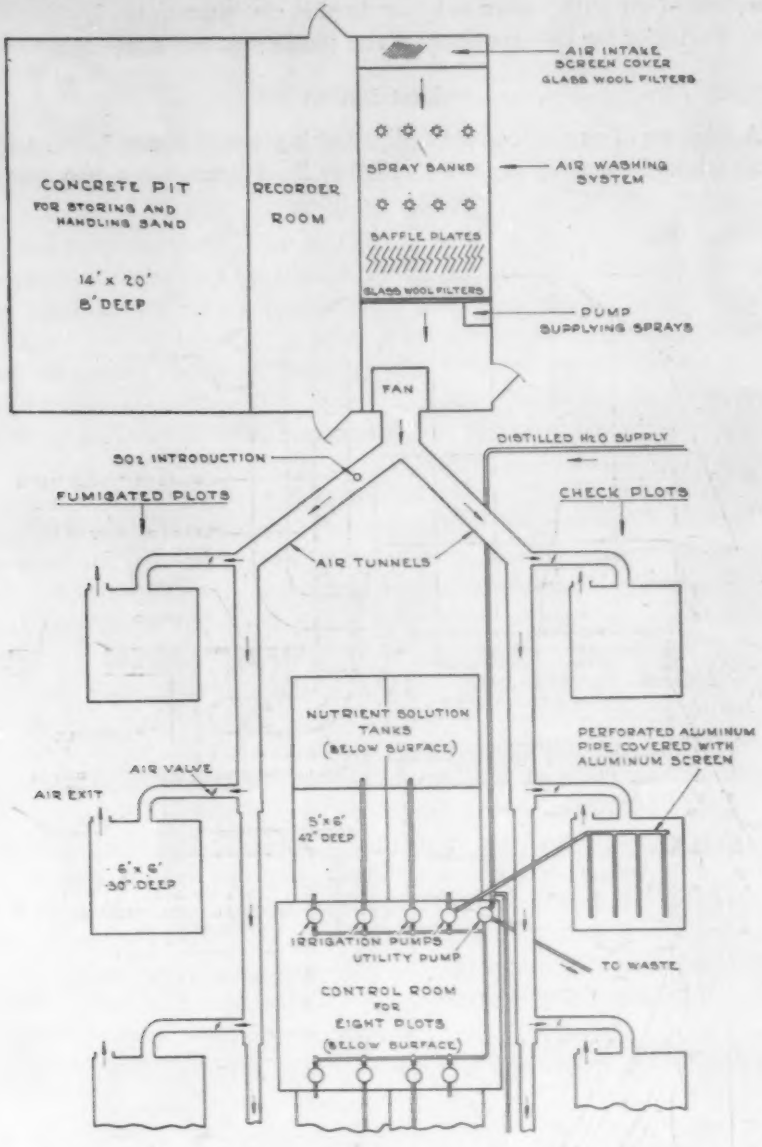


FIG. 2. Diagrammatic plan of the sand-culture and accessory equipment. There are sixteen sand beds and two control rooms.

The sand beds are concrete boxes $6' \times 6' \times 2.5'$, set about 27 inches into the ground. The nutrient supply tanks are enclosed concrete boxes $5' \times 6'$

$\times 3.5'$, placed below the sand beds and connected with them by aluminum pipe through time-clock controlled cast iron motor pumps. The insides of all of these boxes were coated with a heavy layer of asphalt tar, but before the 1942 season started the walls of the sand beds were completely covered by plate glass mounted in tar. More recently the walls of the solution tanks were also covered by plate glass. The nutrient solution is introduced at the bottom of the bed through a manifold of aluminum pipe (fig. 10) covered with a fine aluminum screen to exclude the sand, and the overflow liquid is returned from the top of the sand to the supply tank. A second overflow is provided so that the water can be drained off to waste at the top of the bed when it is desired to wash the sand. A sample of the initial overflow liquid is collected automatically. The solution is allowed to overflow for 5 to 10

FIG. 3. General view of the installation looking southwest. In foreground is the air-washing room, the recorder room, and the utility sand pit.

minutes before the pump is stopped and the excess water is drained back through the pump, requiring about forty minutes. A satisfactory displacement of the old solution and washing of the sand is thus obtained. One irrigation per day is usually sufficient.

The complete installation (fig. 2) consists of 16 sand beds and their individual supply tanks. Two underground laboratories provide access to the motor pumps (fig. 5) and the accessory equipment, which includes facilities for sampling the solutions, adding concentrated nutrients, handling and mixing the solutions, measuring and making up evaporation losses. Air pumps are also provided to force air either up or down through the sand in connection with sulphur dioxide fumigations and root respiration measurements.

On the smooth top edge of each sand box is placed a gastight greenhouse (fig. 6), made of commercial steel window frames, mounted on an angle iron framework. The joint with the concrete surface is made with mastic. This greenhouse has an 8″ intake pipe leading up the north side and entering near the top. A similar outlet pipe emerges from the bottom. For ready access to the inside, the greenhouse is provided with four 10″ × 14″ doors, one on each side, and also one gastight door large enough to admit a man. The whole structure can be removed from the sand bed with a crane (fig. 7).

The ventilation system includes air washing and filtering equipment (fig. 3). The air is washed with tap water in a concrete chamber 7′ × 7′

FIG. 4. View of the plant chambers containing alfalfa on April 29, 1941.

× 14′, using a double bank of 100 sprays, operated by a 3 H.P. motor pump. The spray is removed by the usual galvanized iron eliminator plates, and the air is filtered through glass wool filters before and after entering the spray chamber. A 9,000 c.f.m. blower then delivers the air to the 16 greenhouses through underground tile pipes. Dampers and air valves are provided to control the air flow so that it is equally distributed to the different greenhouses. No attempt has been made to thermostat the plant chambers, but their temperature usually is the same as, or a little below, outside air temperature. Continuous analysis of the washed air has failed to show a detectable amount of sulphur dioxide. The limit of this analytical method is about 2 parts per billion.

Ottawa (Illinois) silica sand is used in the beds. This material consists of clear well-rounded grains, 0.5–0.6 mm. in diameter, assaying 99.8 per cent. silica. The remaining 0.2 per cent. is nearly all iron and aluminum

oxides. It has proven to be entirely satisfactory as to particle size, and pure enough for most purposes.

Each bed contains 4,000 kg. of sand. In an experiment to determine the amount of moisture held by the sand, a bed was flooded and allowed to drain for 2 hours. On sampling with a tube, 11.4 per cent. of moisture was found. After 24 hours, the sand retained 9.4 per cent. water. When air was drawn down through the bed over night after irrigating, 6.9 per cent.

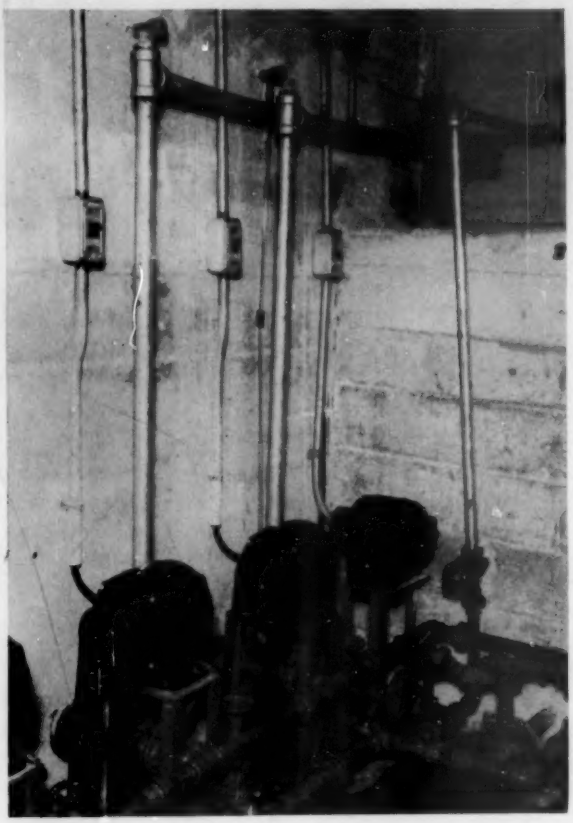


FIG. 5. Solution pumps showing interconnections and discharge pipes to the sand beds.

water was found next day, and 4.8 per cent. after about 1 week. The permanent wilting percentage of this sand has not been determined, but it is probably less than 2 per cent. The bed will, therefore, retain at least 100 to 350 liters of readily available moisture, under different conditions. The maximum amount of evaporation that has been observed with a large crop of alfalfa was about 30 liters per day. This would represent less than 10 per cent. of the water available if the sand were irrigated daily.

At harvest, the root systems can be removed nearly intact if desired. Figure 9 is a photograph of an alfalfa root partially washed free of sand. Figure 10 shows the intermeshed roots of three winter wheat plants and one barley plant, which have been washed free of sand in place. Note part of the irrigation manifold in the bottom of the pit. The tarred wall of the pit in figure 9 may be compared with the plate glass wall in figure 10. If the roots are not desired intact, it is simpler to pull out the larger portions and

FIG. 6. Greenhouse unit, showing access to inside and the strips of sheet aluminum covering sand.

separate the hair roots from the sand by means of a large screen, agitated in the surface of a pool of water (fig. 8). After further washing, the sand can be used again. One prolonged washing with 0.1 N nitric acid has usually been employed. It has been possible to secure a satisfactory control of green algae by the use of strips of sheet aluminum between the plants.

Nutrient solutions

The principal constituents of the nutrient solutions have had approximately the proportions but not more than half the concentrations suggested by HOAGLAND (7). It has been necessary also to make modifications

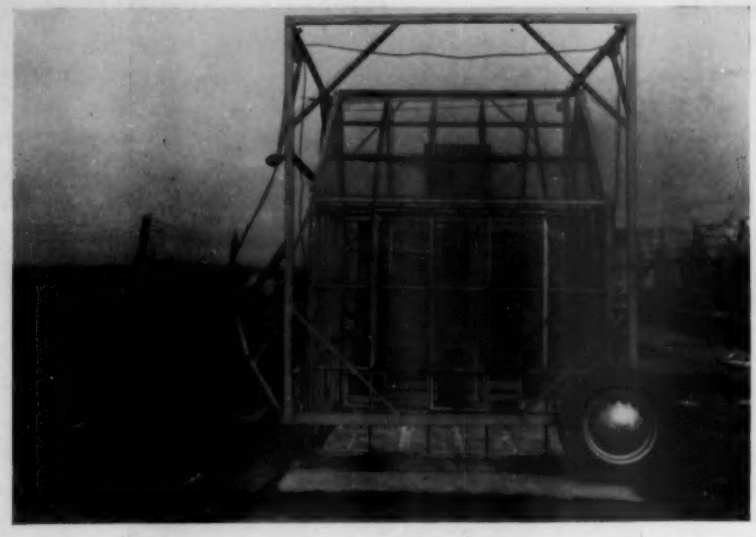


FIG. 7. Greenhouse unit surmounted by the crane for removing the house from the sand pit.

to secure the desired range of sulphur and hydrogen ion concentrations. In general the basic solution has been as follows:

$Ca(NO_3)_2$	0.002 M
KNO_3	0.002 M
$Mg(NO_3)_2$	0.0008 M

To this solution, phosphoric acid or potassium phosphate is added to give a phosphate concentration of 0.0001 M or about 3 p.p.m. More recently the

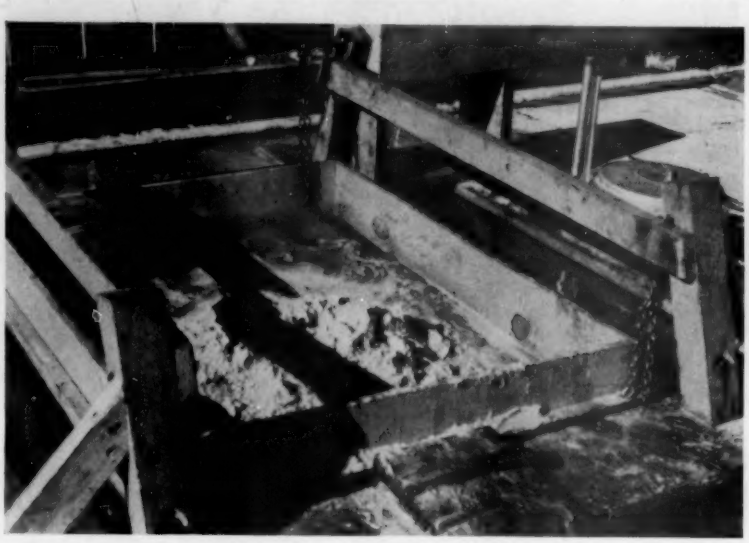


FIG. 8. Equipment for screening the sand to separate the hair roots from the sand.

FIG. 9. Alfalfa root partially washed free of sand (1941).

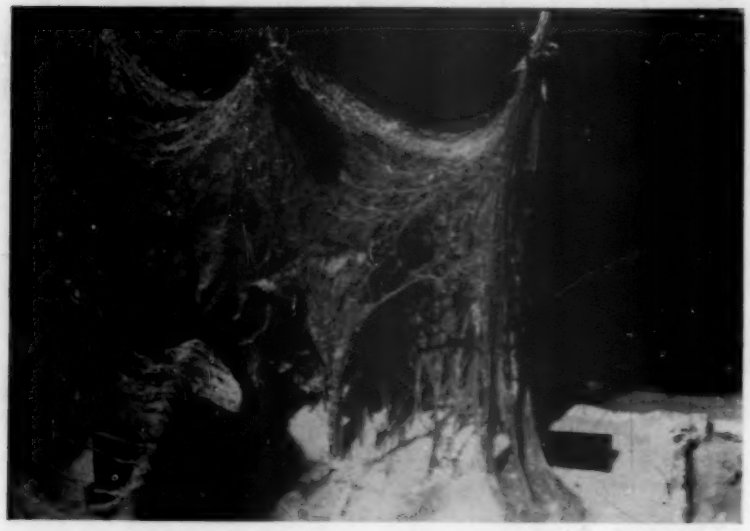


FIG. 10. Roots of four grain plants washed free of sand in place (1942). Note part of irrigation manifold in bottom of the pit.

concentration of the potassium nitrate has been increased to 0.004 M and that of the calcium nitrate reduced to 0.0012 M in accordance with the suggestion of ARNON and HOAGLAND (1) that the ions are absorbed by the plants in about these proportions.

Ammonia or potassium hydroxide is then used to adjust the pH, which is subsequently maintained by the proper nitrate-ammonia ratio as suggested by TRELEASE and TRELEASE (10). It has not been practicable to maintain a phosphorous concentration higher than 3 p.p.m. on account of precipitation. Metaphosphate was tried and seemed promising for a time, but it soon reverted to orthophosphate and precipitated. The desired amount of sulphate is added as potassium, magnesium, or calcium salt. Iron is maintained between 0.15 and 0.5 p.p.m. by repeated additions of ferric nitrate, and the usual "trace" elements are also added. Lower nutrient levels have been secured by dilution of the basic solution without a corresponding dilution of the sulphur, phosphorus, iron, and trace elements. It is necessary to make up these solutions with distilled water, since the best natural water contains too much sulphur for these studies. A 20-gallon-per-hour still and a 3,000-gallon storage tank, provide the large amount of water needed.

The installation has been in use since 1939. Subsequent papers in this series will describe the experiments conducted in it.

Summary

A large sand-culture installation is described, with automatic equipment for subirrigating the beds and facilities for measuring transpiration, photosynthesis, and root respiration. The beds are surmounted by airtight individual greenhouses, supplied with washed and filtered air free from sulphur dioxide, thus permitting control of the gaseous environment.

DEPARTMENT OF AGRICULTURAL RESEARCH
AMERICAN SMELTING AND REFINING COMPANY
SALT LAKE CITY, UTAH

LITERATURE CITED

1. ARNON, D. I., and HOAGLAND, D. R. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. **50**: 463–485. 1940.
2. BIEKART, H. M., and CONNORS, C. H. The greenhouse culture of carnations in sand. New Jersey Agr. Exp. Sta. Bull. 588. 1935.
3. CHAPMAN, H. D., and LIEBIG, G. F. Adaptation and use of automatically operated sand culture equipment. Jour. Agr. Res. **56**: 73–80. 1938.
4. EATON, FRANK M. A large sand culture apparatus. Soil Sci. **31**: 235–241. 1931.
5. ———. Automatically operated sand-culture equipment. Jour. Agr. Res. **53**: 433–444. 1936.

6. ————————. Plant culture equipment. Plant Physiol. **16**: 385–392. 1941.
7. Hoagland, D. R. Optimum nutrient solutions for plants. Science n.s. **52**: 562–564. 1920.
8. Thomas, Moyer D., and Hill, Geo. R. The continuous measurement of photosynthesis, respiration, and transpiration of alfalfa and wheat growing under field conditions. Plant Physiol. **12**: 285–307. 1937.
9. ————————, and ————————. Relation of sulphur dioxide in the atmosphere to photosynthesis and respiration of alfalfa. Plant Physiol. **12**: 309–383. 1937.
10. Trelease, Sam F., and Trelease, Helen M. Physiologically balanced culture solutions with stable hydrogen-ion concentration. Science n.s. **78**: 438–439. 1933.
11. Withrow, R. B., and Biebel, J. P. A subirrigation method of supplying nutrient solutions to plants growing under commercial and experimental conditions. Jour. Agr. Res. **53**: 693–701. 1936.
12. ————————, and ————————. Nutrient solution methods of greenhouse crop production. Purdue University Agr. Exp. Sta. Circular 232. 1937.

THE UTILIZATION OF SULPHATE AND SULPHUR DIOXIDE FOR THE SULPHUR NUTRITION OF ALFALFA[1]

MOYER D. THOMAS, RUSSEL H. HENDRICKS, T. R. COLLIER, AND GEO. R. HILL

(WITH FIVE FIGURES)

Introduction

Though sulphur has long been recognized as an essential element in plants, particularly as a constituent of the proteins, it has not received much consideration in fertilizer studies. As shown by the work of HART and PETERSON (**3**), this was partly due to the older practice of determining sulphur in the ash of vegetation, which gave low results and did not indicate the true sulphur needs of the plants. In practical agriculture, sulphur deficiency has probably been uncommon, at least during the last century, because of important additions of the element from the atmosphere, and important, though unintentional, additions in commercial fertilizers.

Regions of sulphur deficiency, however, have been shown to exist in the Pacific Northwest (**6**), in Western Canada, and in Northern Minnesota (**2**). The location of these regions where winds from industrial areas seldom blow, seems to emphasize the importance of atmospheric sulphur in supplying the needs of those regions in which there is industrial activity and favorable prevailing winds. ALWAY (**1**) has given an historical review of sulphur in agriculture in his presidential address to the American Society of Agronomy, in which this viewpoint was advanced and in which it was suggested that part of the sulphur requirements of plants might be supplied by direct absorption of sulphur dioxide into the leaves, as well as indirect absorption after the gas has been taken into the soil and changed to sulphate. SETTERSTROM, ZIMMERMAN, and CROCKER (**8**) have submitted evidence that sulphur dioxide can increase the growth rate of sulphur-deficient plants.

In this paper a study has been made, using the large sand-culture installation (**9**), of the sulphur needs of alfalfa, and the possibility has been explored of substituting sulphur dioxide for sulphate as the source. The experiments were planned to determine the effect of sublethal concentrations of sulphur dioxide on the growth of alfalfa under a wide range of conditions—such as different hydrogen ion concentrations and different concentrations of the principal nutrient elements. In particular, the effects of sulphur dioxide on sulphur-deficient vegetation were sought.

Methods

1939 EXPERIMENTS

Twelve plots of alfalfa were studied in 1939. Seedling plants were grown in the greenhouse in complete nutrient solutions in silica sand in

[1] This is the fourth of a series of papers on "The Effect of Prolonged Low Concentrations of Sulphur Dioxide upon Plants."

deep narrow wooden boxes 4″ × 30″ by 18″ deep. Seed of Turkestan alfalfa, derived from a single plant selection, and furnished by the Utah Experiment Station, was planted in February. The plants were topped in April and prepared for transplanting by removing one side and the bottom of each box and washing away the sand with a spray of water. The seedlings were sorted and those of uniform size were distributed on trays so that all the plots received 25 plants which were as nearly the same size as possible. The average fresh weight of the seedlings was about 5 grams per plant; dry weight about 0.7 grams per plant. The transplanting was completed by inserting an 18″ metal planting tool enclosing the seedlings into the moist sand. The tool was then separated into two parts and removed, leaving the seedling in place. These transplanting operations were carried out with negligible injury to the plants.

All the plots were supplied with a base nutrient solution of the approximate composition already indicated (**9**). Six of the plots were maintained at a pH of about 5.5 and the other six at about pH 7.0. Four plots had no sulphur added intentionally to the nutrient solution; four had sulphate equivalent to 5 p.p.m. of sulphur; and four 90 p.p.m. of sulphur. Thus there were six pairs of plots with low and high pH and with low, medium, and high sulphate in the nutrient solution. One plot of each pair was fumigated with sulphur dioxide at a concentration of about 0.1 p.p.m. for 6 to 7 hours each day (usually from 9 A.M. to 4 P.M.) and six days each week throughout the growth period. The other plot of each pair was unfumigated. Four crops were grown during the season, and after cutting the fourth crop, the roots were also harvested.

1940–1941 EXPERIMENTS

Seedling alfalfa plants, grown as in 1939 were transplanted on April 25, 1940 to 12 plots of new sand and 4 plots of used sand that had been washed and steam sterilized. The seedlings had been treated with Rhizobium, supplied by the University of Wisconsin, and were nodulated when transplanted.

An attempt was made after transplanting the seedlings to grow one-half the plots in a solution 40 per cent. of the strength of the 1939 solution, and the other half in a solution of 20 per cent. strength. The plants did not grow as well as in 1939 and it was decided on May 8 to raise the concentration of the former group 2.5 fold. The weaker solutions were not modified until June 3, when one millimol of calcium nitrate was added to them, because the plants appeared to be very unthrifty. This group of plots was placed on the 1939 level of nutrient concentration for the second crop and maintained at that level throughout the remainder of the experiment. The other plots were also maintained at this level for the second crop in 1940; thereafter the concentration of the solution was progressively lowered until it finally reached 3 per cent. of this value. The pH of all the solutions was maintained at about 5.5 during the two years. Four levels of sulphate concentration were established in the nutrient solutions. Four plots had

no sulphate added intentionally. The other three groups had nominal sulphate concentrations equivalent to 0.8 p.p.m., 1.5 p.p.m., and 10 p.p.m. sulphur. There were, therefore, 8 pairs of plots with low and high nutrient levels and low, medium low, medium, and high sulphate sulphur in the nutrient solution. It should be noted that these designations of the sulphate concentrations do not have the same values as in 1939. One plot of each pair was fumigated with sulphur dioxide as in 1939, the other plot being untreated. Four crops were harvested in 1940; then five crops and finally the roots in 1941.

Results

Cultural data

Average concentrations of the principal nutrients, determined by analysis of the solutions, for each crop in 1939 are summarized in table I and the corresponding data for 1940–1941 are given in table II. These tables include the fumigation data. In the 1940–1941 data, the "low nutrient level" group of plots for the first crop in 1940, became the "high nutrient level" group for the second and subsequent crops and vice versa. It is particularly interesting to note the low nutrient concentrations employed in 1941, on which good growth was obtained, due probably to the well-established root systems. Even the highest of these concentrations was inadequate for the first crop in 1940 when the root systems were small.

Growth of the alfalfa was vigorous. Four crops were harvested during each first year and five crops during the second year. The plant chambers were repeatedly filled with vegetation, as already illustrated (**9**). Maximum yields were at the rate of 10 tons per acre of dry alfalfa in 1939, 6.5 tons in 1940, and 15 tons in 1941. The sulphur dioxide fumigations did not cause any acute markings, but chlorotic markings usually developed on the older leaves to the extent of about 5 to 10 per cent. of the leaf area of the plot as described later.

Table III gives the chlorophyll content of all the crops, determined on fresh leaves by the method of Schertz (**7**). These leaf samples were obtained by separating the leaves and stems of a large representative portion of the plots at harvest, as already described (**11**). The table confirms the visual observations, that the leaves of the sulphur-deficient plants were not as dark green in color as the plants supplied with adequate sulphur, either from the nutrient solution or from sulphur dioxide. The "low sulphur-high nutrient" check plot particularly, had at all times a distinctly yellow cast. An exception was the "high sulphur-low nutrient" fumigated plot, which became chlorotic on the last three crops in 1941, due possibly to the development of root disease.

Considerable difficulty was experienced after the first crop in 1939 due to aphis, which were sufficiently numerous in a few cases to reduce somewhat the yield of the crops. Thrips also were present. In 1940–1941 aphis were completely eliminated by the nicotine sprays used to control the more

TABLE I

CULTURAL DATA FOR 1939 EXPERIMENTS

CROP	DATES HARVESTED	pH		SULPHATE CONCENTRATION			SO_2 FUMIGATION		AVERAGE NUTRIENT CONCENTRATION							
															NH_3	
		HIGH	LOW	HIGH	MEDIUM	LOW	TOTAL TIME	CONCENTRATION	K	Ca	Mg	P	Fe	NO_3	HIGH pH	LOW pH
				p.p.m. S			*hours*	*p.p.m.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*
1st	June 21–22	6.73	5.52	83.0	3.9	0.85	293	0.11	3.5	3.2	1.15	0.25	0.008	8.9	0.04	0.56
2nd	July 26–27	7.06	5.58	88.0	4.2	0.61	179	0.10	3.4	2.8	1.18	0.18	0.007	8.2	0.00	0.34
3rd	Sept. 6– 7	7.05	5.69	88.0	4.4	0.46	225	0.10	2.9	2.4	0.94	0.08	0.005	8.1	0.00	0.22
4th	Oct. 26–27	7.04	5.65	80.0	3.6	0.26	223	0.09	2.5	2.5	1.06	0.04	0.003	6.8	0.14	0.74

TABLE II

CULTURAL DATA FOR 1940 AND 1941 EXPERIMENTS

Crop	Date harvested	Sulphate concentration				SO_2 fumigation		Average nutrient concentration						
		High	Medium	Medium-low	Low	Total time	Concentration	K	Ca	Mg	P	Fe	NO_3	NH_3
		p.p.m. S				*hours*	*p.p.m.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*	*m. mol.*
						High nutrient level								
						1940								
1st	July 1	10.9	2.24	0.93	0.75	240.0	0.09	2.69	2.42	1.16	0.06	0.005	8.00	1.19
2nd	Aug. 9	10.9	2.13	0.90	0.53	182.0	0.11	2.24	1.76	0.70	0.04	0.007	7.30	0.82
3rd	Sept. 17	8.8	1.28	0.61	0.48	167.0	0.12	2.33	1.78	1.09	0.05	0.008	7.30	0.17
4th	Nov. 13	8.4	1.21	0.61	0.36	347.0	0.12	1.77	1.52	0.85	0.03	0.003	5.40	0.07
						1941								
1st	May 7	8.8	1.36	0.53	0.14	318.0	0.13	1.21	1.58	0.73	0.02	0.006	6.15	0.53
2nd	June 12	9.8	1.45	0.57	0.18	105.0	0.12	1.70	1.84	0.99	0.02	0.003	7.30	0.64
3rd	July 22	9.1	1.40	0.53	0.21	137.0	0.09	1.56	1.91	0.99	0.01	0.005	6.85	0.26
4th	Sept. 2	8.9	1.38	0.53	0.25	150.0	0.08	2.26	1.83	0.85	0.02	0.003	7.30	0.52
5th	Oct. 16	8.6	1.26	0.45	0.26	115.0	0.05	2.19	1.73	0.77	0.01	0.005	6.43	0.07
						Low nutrient level								
						1940								
1st	June 28	11.1	2.42	0.84	0.46	240.0	0.09	1.49	0.41	0.29	0.05	0.005	1.00	0.15
2nd	Aug. 8	9.0	1.16	0.61	0.35	182.0	0.11	2.88	1.84	1.00	0.06	0.008	8.30	1.04
3rd	Sept. 16	9.0	1.19	0.39	0.39	167.0	0.12	0.90	0.67	0.41	0.05	0.008	2.72	0.15
4th	Nov. 12	7.5	1.34	0.60	0.33	347.0	0.12	0.24	0.27	0.26	0.04	0.006	1.03	0.08
						1941								
1st	May 6	9.1	1.46	0.49	0.19	318.0	0.13	0.08	0.19	0.04	0.02	0.006	0.56	0.12
2nd	June 11	10.3	1.66	0.59	0.18	105.0	0.12	0.21	0.22	0.11	0.03	0.004	0.79	0.11
3rd	July 21	9.1	1.35	0.56	0.22	137.0	0.09	0.11	0.21	0.08	0.03	0.006	0.60	0.04
4th	Sept. 1	8.9	1.65	0.66	0.28	150.0	0.08	0.09	0.12	0.06	0.02	0.004	0.26	0.05
5th	Oct. 15	9.0	1.78	0.56	0.31	115.0	0.05	0.09	0.10	0.03	0.01	0.006	0.19	0.01

TABLE III

CHLOROPHYLL CONTENT OF THE ALFALFA LEAVES

Crop	High S		Medium S		Medium-low S		Low S		High S		Medium S		Medium-low S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
			High pH				1939				Low pH					
1st	1.92	1.97	1.60	1.90	.....	.....	1.50	1.70	2.03	1.93	1.90	1.98	.....	.....	1.64	1.74
2nd	2.21	2.23	2.10	2.14	.....	.....	1.91	2.10	2.30	2.27	2.30	2.10	.....	.....	1.90	2.25
3rd	2.20	2.20	2.12	2.05	.....	.....	1.82	2.02	2.31	2.15	2.37	2.24	.....	.....	1.90	2.25
4th	2.12	1.76	2.03	1.82*	.....	.....	1.32	1.79	2.04	2.07	2.12	1.91*	.....	.....	1.32	1.83
Average	2.11	2.04	1.96	1.98	.....	.....	1.64	1.90	2.17	2.11	2.17	2.06	.....	.....	1.69	2.02
			High nutrient				1940				Low nutrient					
1st	1.31	1.31	1.58	1.47	1.41	1.52	1.26	1.57	1.22	1.31	1.37	1.52	1.41	1.47	1.31	1.30
2nd	2.04	1.90	1.90	2.10	1.60	1.82	1.39	1.58	1.85	1.92	2.00	1.92	1.52	1.71	1.47	1.80
3rd	2.15	2.44	2.28	2.13	2.17	1.93	1.90	2.02	2.40	2.19	2.12	2.03	2.11	2.09	1.93	2.05
4th	1.50	1.55	1.69	1.50	1.55	1.41	1.39	1.40	1.51	1.44	1.35	1.35	1.67	1.46	1.52	1.56
Average	1.75	1.80	1.86	1.80	1.68	1.68	1.48	1.64	1.74	1.71	1.71	1.71	1.68	1.67	1.56	1.68
			High nutrient				1941				Low nutrient					
1st	1.52	1.95	2.01	2.00	1.96	1.92	1.49	1.79	1.84	1.77	1.67	1.67	1.61	1.50	1.49	1.45
2nd	1.71	1.65	2.13	1.91	1.81	1.58	1.01	1.60	1.85	1.67	1.77	1.67	1.77	1.45	1.39	1.69
3rd	1.45	1.37	1.46	1.58	1.37	1.35	0.90	1.20	1.34	1.20	1.37	1.35	1.10	1.06	1.07	1.09
4th	2.10	2.03	2.10	2.06	1.99	1.76	1.29	1.84	1.77	1.18	1.65	1.47	1.29	1.46	1.55	1.40
5th	1.83	1.82	1.84	1.83	1.84	1.75	1.05	1.36	1.43	1.17	1.74	1.64	1.55	1.44	1.44	1.06
Average	1.72	1.76	1.91	1.88	1.79	1.67	1.15	1.56	1.64	1.40	1.64	1.56	1.46	1.38	1.39	1.34

* Heavy infestation of aphis.

troublesome thrips. It is probable that thrips were responsible for some of the variability in the data to follow. Infestation was heavy and general and it was necessary to reduce their population at the beginning of each crop by nicotine sprays, because they attacked the growing points and interfered seriously with the development of the new shoots. It was im-

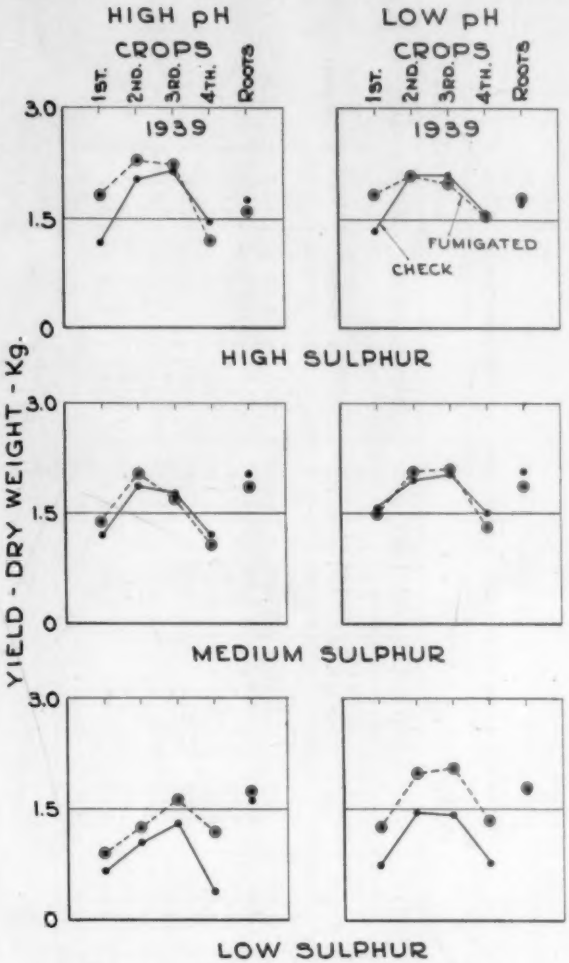


FIG. 1. Alfalfa crop yield data for 1939.

practicable to spray the larger plants, but the thrips seemed to be much less harmful at this stage. The nicotine sprays produced some typical foliar markings, which destroyed about 1 per cent. of the leaf area of all the plots.

YIELD

Yield data are presented as kilograms dry weight per plot for the 1939 crops in table IV and figure 1 and for the 1940–1941 crops in table V and figure 2. The yearly yields are summarized in table VI and figure 3. The

TABLE IV

YIELD DATA FOR 1939 EXPERIMENTS (DRY WEIGHT PER PLOT)

Crop	High pH (7.0)						Low pH (5.5)					
	High sulphur		Medium sulphur		Low sulphur		High sulphur		Medium sulphur		Low sulphur	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
	kg.	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*
1st	1.17	1.82	1.20	1.35	0.66	0.91	1.35	1.86	1.56	1.50	0.72	1.25
2nd	2.05	2.31	1.88	2.03	1.05	1.25	2.11	2.11	1.96	2.03	1.48	1.98
3rd	2.16	2.22	1.78	1.72	1.30	1.65	2.10	1.98	2.07	2.08	1.43	2.07
4th	1.44	1.19	1.20	1.07*	0.39	1.21	1.56	1.53	1.51	1.32*	0.77	1.35
Total crops	6.82	7.54	6.06	6.17	3.40	5.02	7.12	7.48	7.10	6.93	4.40	6.65
Roots	1.19	1.07	1.45	1.27	1.20	1.20	1.10	1.17	1.37	1.08	1.22	1.20
Crowns	0.58	0.55	0.62	0.59	0.43	0.55	0.61	0.58	0.70	0.59	0.52	0.58
Roots and crowns	1.77	1.62	2.07	1.86	1.63	1.75	1.71	1.75	2.07	1.67	1.74	1.78
Total	8.59	9.16	8.13	8.03	5.03	6.77	8.83	9.23	9.17	8.60	6.14	8.43

* Heavy infestation of aphis.

TABLE V

YIELD DATA FOR 1940–1941 (DRY WEIGHT PER PLOT)

CROP	HIGH NUTRIENT LEVEL								LOW NUTRIENT LEVEL							
	HIGH S		MEDIUM S		MEDIUM-LOW S		LOW S		HIGH S		MEDIUM S		MEDIUM-LOW S		LOW S	
	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED
	kg.	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*
								1940								
1st*	0.52	0.60	0.62	0.50	0.37	0.50	0.41	0.63	0.16	0.12	0.07	0.14	0.08	0.08	0.04	0.09
2nd	1.11	0.97	0.86	1.12	0.53	0.76	0.34	0.40	1.11	1.04	1.29	1.08	0.54	1.19	0.47	0.86
3rd	1.61	1.59	1.50	1.45	1.05	1.28	0.66	1.12	1.41	1.31	1.43	1.29	1.04	1.46	0.69	1.25
4th	1.63	1.49	1.44	1.20	1.06	1.21	0.58	1.14	0.94	0.86	0.95	0.84	0.85	0.95	0.55	1.02
Total	4.87	4.65	4.42	4.27	3.01	3.75	1.99	3.29	3.62	3.33	3.74	3.35	2.51	3.68	1.75	3.22
								1941								
1st	3.07	2.97	3.22	2.97	2.99	3.19	2.59	2.82	2.51	2.03	2.34	2.28	2.29	2.17	2.20	2.42
2nd	1.98	1.74	1.97	1.96	1.80	1.78	1.20	1.45	1.60	1.41	1.59	1.44	1.30	1.40	1.12	1.31
3rd	2.54	2.30	2.57	2.63	2.06	2.27	1.39	1.82	1.93	1.74	2.04	1.94	1.55	1.60	1.27	1.59
4th	2.42	2.07	2.08	2.12	1.90	2.00	1.31	1.70	1.24	0.98†	1.22	1.17	0.89	1.05	1.26‡	0.96
5th	1.31	1.23	1.14	1.21	1.16	1.13	0.74	0.81	0.73	0.51†	0.87	0.84	0.51	0.56	0.73‡	0.50
Total	11.32	10.31	10.98	10.89	9.91	10.37	7.23	8.60	8.01	6.67	8.06	7.67	6.54	6.78	6.58	6.78
Total crops	16.19	14.96	15.40	15.16	12.92	14.12	9.22	11.89	11.63	10.00	11.80	11.02	9.05	10.46	8.33	10.00
Roots	2.08	2.33	2.42	2.33	2.38	1.95	2.23	2.20	2.47	2.15†	2.47	2.29	2.59	2.38	2.65	2.33
Crowns	0.80	0.74	0.76	0.68	0.80	0.75	0.63	0.52	0.65	0.59	0.72	0.59	0.66	0.58	0.60	0.57
Roots and crowns	2.88	3.07	3.18	3.01	3.18	2.70	2.86	2.72	3.12	2.74	3.19	2.88	3.25	2.96	3.25	2.90
Total crops and roots	19.07	18.03	18.58	18.17	16.10	16.82	12.08	14.61	14.75	12.74†	14.99	13.90	12.30	13.42	11.58	12.90

* The yield data for low nutrient level, first crop 1940, were obtained from the plots from which all subsequent high nutrient level yield data were obtained, and vice versa.

† 4th and 5th crops were seriously affected by root disease.

‡ 4th and 5th crops had a nearly adequate supply of sulphur from an unknown source.

1939 data indicate a somewhat better growth of alfalfa at pH 5.5 than at pH 7, at the "low" and "medium" sulphur levels. At the "high sulphur level" the pH effect seemed to disappear. The reduced yield of the check

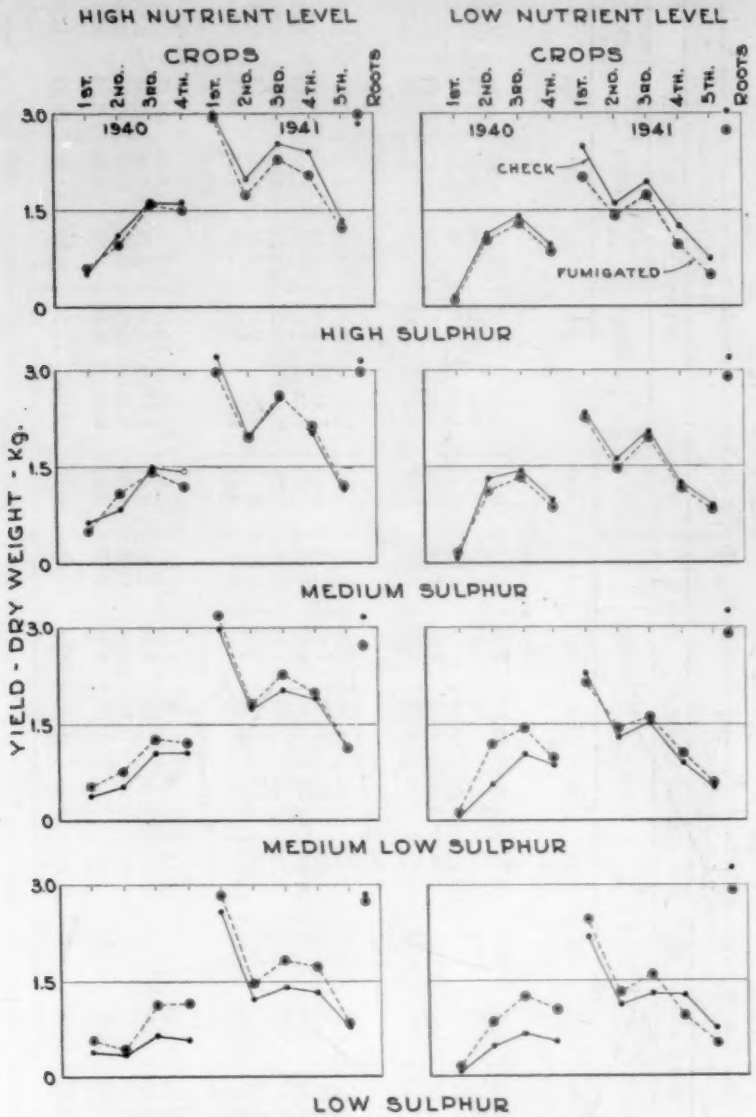


Fig. 2. Alfalfa crop yield data for 1940 and 1941.

plots at the low sulphur level was a definite indication of a deficient supply of this element. In most cases growth with "medium sulphur" was about the same as with high sulphur. The sulphur dioxide fumigations increased the yield markedly on all the low sulphur plots, but had little effect on the others, except for the anomalous increase of the first crop with high sulphur

for which no explanation is apparent. Two of the medium sulphur fumigated crops gave lower yields than the checks due to heavy infestations of aphis.

The 1940–1941 yield data (table V, fig. 2) indicate a sulphur deficiency in the low sulphur and medium-low sulphur groups of plots. In nearly all of these crops, the check yields were lower than the corresponding yields with medium sulphur and high sulphur and further, the fumigated plots almost invariably gave higher yields than the corresponding checks. The exception was the low nutrient, low sulphur check plot which on the fourth and fifth crops in 1941 not only gave larger yields than its fumigated mate,

TABLE VI

RATIOS OF THE AVERAGE YEARLY YIELDS OF THE FUMIGATED PLOTS TO THE CORRESPONDING CHECK PLOTS

	High S	Medium S	Medium-low S	Low S	High S	Medium S	Medium-low S	Low S
				1939 data				
		High pH				Low pH		
Crops	1.11	1.02	.....	1.48	1.05	0.98	.....	1.51
Roots	0.90	0.88	.....	1.00	1.06	0.79	.....	0.98
Roots + crowns	0.92	0.90	.....	1.07	1.03	0.81	.....	1.02
Total	1.07	0.99	.....	1.35	1.05	0.94	.....	1.37
				1940 data				
		High nutrient				Low nutrient		
Crops	0.96	0.97	1.25	1.65	0.92	0.90	1.47	1.84
				1941 data				
		High nutrient				Low nutrient		
Crops	0.91	0.99	1.05	1.19	0.84*	0.95	1.04	1.03†
Roots	1.12	0.98	0.82	0.99	0.87*	0.93	0.92	0.88
Roots + crowns	1.07	0.94	0.85	0.95	0.88*	0.93	0.90	0.89
Total 1940–41	0.95	0.98	1.05	1.21	0.87*	0.93	1.09	1.11‡

* One plant dead and several others with root disease at final harvest.
† Omitting fourth and fifth crops, ratio = 1.16 (see table V).
‡ Omitting fourth and fifth crops (1941), ratio = 1.19 (see table V).

but also exceeded the yields of the medium-low sulphur plots and even equalled the yields of the medium sulphur and high sulphur plots. Analytical data to follow, indicate that these two crops received a nearly adequate supply of sulphur from some source other than the nutrient solution, possibly from the tar-covered walls of the sand bed, or from the sand itself. A careful examination of this container did not reveal any breaks in the tar lining or any other unusual appearance. It is difficult to understand how there could have been a sudden increase in the sulphur supply from the sand. No leaks were found in the system and the nutrient solutions did not show an appreciable increase in sulphate content.

In 1940–1941, as in 1939, the fumigated low sulphur and medium-low sulphur plots gave yields that were intermediate between the corresponding check yields and the largest yields obtained with adequate sulphur in the nutrient solution. This suggests that sulphur dioxide, supplied to the leaves for sulphur nutrition, is not as efficient as sulphate supplied to the roots. Presumably, this is due in part to the fact that sulphur dioxide, after absorption and oxidation to sulphate in the leaves, tends to remain in the leaves and is only slowly translocated to other parts of the plant.

The attempt was made in 1940–1941 to prevent the absorption by the sand of sulphur dioxide which would subsequently be oxidized to sulphate and absorbed by the roots. For this purpose, a stream of air (0.4 cubic ft. per minute) was forced upward through the sand throughout the fumi-

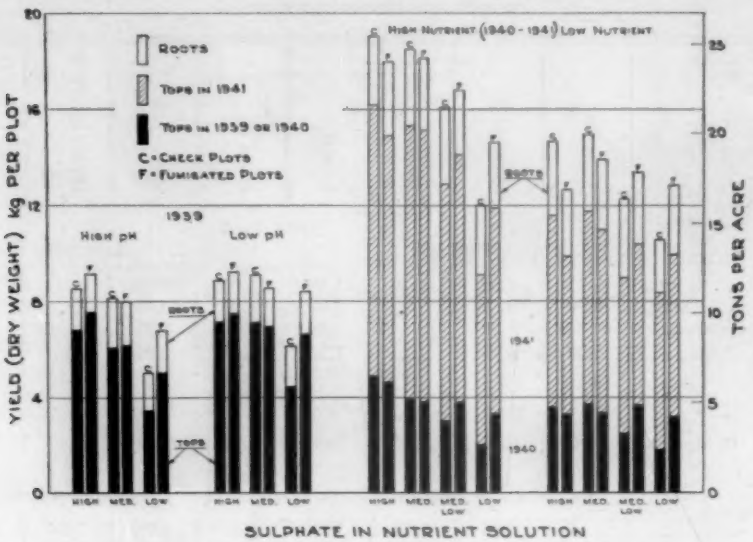


FIG. 3. Total yearly yields of alfalfa, including roots, for 1939, 1940, and 1941.

gation period. The check plots were similarly treated. This procedure was not adopted in 1939. Absorption of sulphur dioxide by the sand was never entirely excluded. Analysis of the nutrient solution indicated that its sulphur content was probably raised about 0.1 p.p.m. by this means in 1940–1941, but this increase seemed to have very little effect on the experiment.

The yield data suggest that the low sulphur and medium-low sulphur plots were much less deficient in sulphur in 1941 than in 1939 or 1940. In fact the yield of the large first crop in 1941 was nearly independent of the nominal sulphur supply. This was probably due to the long period during the winter when the plants could accumulate an appreciable amount of sulphur by slow absorption from the walls or elsewhere and also to the large root system in 1941. Further, it was impossible to operate the air-

washing system during the cold weather, and some sulphur was doubtless derived from the atmosphere at this time.

It will be noted that the weights of the different root systems did not parallel the variations in weight of the tops. In 1939 the medium sulphur check plots had slightly larger roots than the others; but the low sulphur roots were a little larger than the high sulphur roots. In all cases in 1939, except the low pH-high sulphur pair, the check roots were as large as or larger than the corresponding fumigated roots. However, the roots plus crowns were heavier in the check plots in only three of the 6 pairs. In 1941, the check roots and also roots plus crowns, exceeded the corresponding fumigated values in 7 out of 8 cases.

The ratios of yields of corresponding fumigated and check plots are summarized for all 3 years in table VI, and the yearly yields are shown graphically in figure 3. There was an increase in yield due to fumigation with sulphur dioxide in the low sulphur plots of 48 per cent. and 51 per cent. in 1939, and of 65 per cent. and 84 per cent. in 1940. The increase in the medium-low sulphur plots in 1940 was 25 per cent. and 47 per cent. The corresponding increases in 1941 were only 3 per cent. to 19 per cent., though as indicated in table VI, the 3 per cent. value was low due to the fact that the check plot of the pair which gave this result was somehow supplied with an adequate amount of sulphur on the fourth and fifth crops, as shown by analysis. Statistically, all of these increases in yield are highly significant.

The medium sulphur and high sulphur plots had ratios fairly close to unity. The 1939 fumigated plots tended to give a little larger yield than their checks, while the corresponding 1940 and 1941 fumigated plots gave somewhat smaller yields. In order to evaluate the statistical significance of these ratios, it is desirable to group together all the high nutrient plots which had an adequate supply of sulphur. These include the 1939 plots, and there is no apparent reason to make a distinction between those with low or high pH. The average of 8 ratios for the crops—4 in 1939, 2 in 1940, and 2 in 1941—is 1.00 with a standard deviation of 0.06. The average of 6 ratios of the roots—4 in 1939 and 2 in 1941—is 0.95 ± 0.11. These values are not significantly different from unity by the test of FISHER's t. Finally, the average of 6 ratios of total yield, including roots—4 for 1939 and 2 for 1941—is 1.00 ± 0.05. If the individual pairs of crop yields of this group are subjected to the analysis of variance, no significant effect on yield due to fumigation is indicated.

It may, therefore, be concluded from these experiments that the yield of alfalfa growing in a sulphur-deficient medium will be increased by sulphur dioxide fumigations which do not cause appreciable leaf injury, but not increased to the level of non-deficient plants. On the other hand, such fumigations will have no measurable effect at all if the roots are adequately supplied with sulphur and the general nutritional level is adequate. This latter observation is in accord with the results of experiments with alfalfa

grown in soil, previously published (11), and also with similar experiments by KATZ and LEDINGHAM (5). SETTERSTROM, ZIMMERMAN, and CROCKER (8) arrived at similar conclusions, using different nutrient solutions, which supplied both full nutrient and deficient nutrient.

The low nutrient plots in 1940–1941 present a somewhat confused picture. On the one hand, considering the sulphur-deficient plots, the increment of growth of the two fumigated plots with low nutrient was as great as, or even greater than, the increment of the other two fumigated plots with high nutrient. On the other hand, the two low nutrient fumigated plots with adequate sulphate, showed a decreased yield of 7 and 13 per cent. as compared with their checks. Considering the variability of the ratios already discussed, the 7 per cent. reduction in yield may not be significant, and the 13 per cent. reduction occurred on a plot which had some root disease. The yields of these two fumigated plots were similarly low on the second crop in 1940 when the nutrient level was high suggesting that they were inherently poorer plots than their checks. No conclusion can safely be drawn from these limited data concerning the effect of these fumigation treatments on the yield.

It should be noted, however, that the fumigations were not without effect on the leaves. Due to the long continued absorption of sulphur dioxide, which accumulated in the leaves as sulphate, the older leaves became chlorotic and some were eventually shed. The first two crops in 1940 showed this effect to a negligible extent. Subsequent crops showed it appreciably, particularly those that grew slowly at the end of the season. On the medium sulphur-low nutrient fumigated plot, the average shedding of leaves for 7 crops after the second crop in 1940 was 5 per cent. and the average extent of advanced chlorotic markings at harvest was also 5 per cent. of the leaf area. All of the other fumigated plots had approximately these values, except the high sulphur-low nutrient plot which had 6 per cent. of shed leaves and 17 per cent. of advanced chlorotic markings. The check plots had only about 1 per cent. of shed and chlorotic leaves on the average. On the high nutrient plots the amount of leaf destruction evidently did not influence the yield appreciably. Whether these foliar effects would have more influence on the yeld of plants growing in a low nutrient solution than on the yield in a high nutrient solution, needs further study.

PHOTOSYNTHESIS AND RESPIRATION

Two carbon dioxide autometers (10) were operated nearly continuously on some of these alfalfa plots in 1940–1941. Each machine sampled from the intakes and outlets of two plots, with a periodic and automatic interchange of sample sources so that an accurate comparison of the carbon dioxide exchange of the two plots was obtained. One machine was operated on the high sulphur plots; the other on the low sulphur plots. One fumigated plot and its unfumigated check were compared for two days, then the other fumigated plot and its check were measured for two days. Observations on

each crop were commenced as soon as the new growth had developed enough to give measurable activity. The data were calculated and plotted as previously described (**10**). Curves were obtained that were quite similar in appearance to those published earlier. In view of the fact that complete records were not obtained on any of the plots, it was not practicable to calculate a carbon dioxide balance sheet as was done before (**10, 11**). Since these measurements, however, were taken intermittently during the time when most of the crop growth occurred, they may be considered to be representative of the whole growth period. Accordingly, comparisons of the

TABLE VII

RATIOS OF NET ASSIMILATION AND YIELD ON THE FUMIGATED PLOTS TO THE CORRESPONDING VALUES ON THE CHECK PLOTS

Crop	High S High nutrient		High S Low nutrient		Low S High nutrient		Low S Low nutrient	
	Net assimilation	Yield	Net assimilation	Yield	Net assimilation	Yield	Net assimilation	Yield
				1940				
1st	.....	1.15	.....	0.75	.....	1.54	1.37	2.25
2nd	.....	0.88	.....	0.94	1.32	1.18	1.44	1.83
3rd	0.95	0.99	1.01	0.93	1.26	1.70	1.44	1.81
4th	0.98	0.92	0.84	0.92	1.33	1.97	1.29	1.86
				1941				
1st	1.03	0.97	0.90	0.81	1.03	1.09	0.98	1.11
2nd	0.96	0.88	0.97	0.88	1.25	1.21	1.13	1.17
3rd	0.96	0.91	1.00	0.91	1.34	1.31	1.19	1.25
4th	0.95	0.86	0.83	0.79*	1.29	1.30	0.90	0.76†
5th	0.97	0.94	0.68	0.70*	1.15	1.10	0.67	0.69†
				Averages 1940–1941				
Tops	.....	0.92	.....	0.86	.....	1.29	.....	1.20
Roots	.....	1.07	.....	0.88	.....	0.95	.....	0.89
Total	0.97	0.95	0.90	0.87*	1.23	1.21	1.13	1.11

* Root disease developed in fumigated plot during 1941.
† Check plot received sulphur from an unknown source.

net assimilation based on carbon dioxide exchange should parallel the yield data, particularly the total yield, including the roots.

Table VII gives the net assimilation of four fumigated plots divided in each case by the net assimilation of the corresponding check plots. Similar ratios for the yields are also given. Two pairs of plots were high sulphur; the other two low sulphur. In the high nutrient-high sulphur pair of plots the ratios of the net assimilation for the crops were generally larger than the ratios of the crop yields. This was probably due to the fact that the fumigated roots were somewhat larger than the check roots, and therefore a larger proportion of assimilated material went into the fumigated roots, as compared with the tops, thus reducing the ratio of the top weights. The reverse was true of both the low sulphur pairs, particularly in 1940, when

there was a large difference between the ratios of net assimilation and crop yield, indicating that the check roots were receiving a larger proportion of the assimilated material as compared with the tops, than was the case with the fumigated plots. In 1941 these differences were hardly apparent. The low-nutrient-high sulphur pair showed only small differences throughout. The final comparisons between assimilation and total yield, including roots, were very close in all four pairs of plots.

Water requirements

The amount of water used in these experiments was determined for each plot by frequent readings of the gauges on the supply tanks and by careful attention to the distilled water and solution volumes added and sample volumes taken. The water loss was largely transpiration, but there was a measurable amount of evaporation from the surface of the sand, as indicated by the losses which occurred when the amount of vegetation was small. That these losses were not large was due in part to the low water-holding capacity of the sand and in part to the sheet-aluminum cover between the rows of plants. The gauge readings showed that during the first few days of each crop, when transpiration was probably negligible, the plots lost about 1.5 to 4 kilograms of water per day. It is estimated, therefore, that the total water loss from the surface of the sand did not exceed 800 kilograms during each season, and possibly was not more than about 600 kilograms.

The transpiration data are summarized in table VIII, as kilograms of water evaporated per plot. In addition, the amount of water evaporated per unit of dry matter produced is included. The latter values are calculated in two ways: first, from the water losses as measured, and then from these losses less 800 kilograms, to give a minimum value for true transpiration per unit dry matter.

Except for the low sulphur plots, which transpired considerably less water per plot than the others, there was a rather narrow range in the amount of transpiration in each group of plots. Transpiration was not affected by the pH range employed, but it was lowered by lowering the nutrient concentration, except in the low sulphur plots, in which it was independent of the nutrient concentration. It is unlikely that any of the differences in transpiration per plot between check and fumigated pairs are large enough to be significant, except possibly in the low and medium-low sulphur pairs in 1939 and 1940. In the latter cases, the fumigated plots transpired only 5 to 13 per cent. more water than their checks; whereas they had 25 to 84 per cent. more top growth than the checks.

The amount of water transpired in producing unit weight of crops was much higher in the first year crops (1939 and 1940) than in the second year crops (1941). Rather close concordance was noted between the 1939 crops and the comparable high nutrient crops in 1940. There was a much smaller difference, however, between the 1939 crops plus roots and the com-

TABLE VIII

WATER REQUIREMENTS OF THE 1939, 1940, 1941 CROPS

Year	High pH 1939 or high nutrient 1940–1941						Low pH 1939 or low nutrient 1940–1941							
	High S		Medium S		Medium-low S		Low S		High S		Medium S		Medium-low S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
Kilograms of water transpired per plot																
1939	2610	2720	2560	2620	.....	.....	2120	2290	2550	2610	2760	2620	.....	.....	2210	2490
1940	1980	1970	1960	1850	1810	1930	1660	1740	1790	1880	1800	1780	1690	1820	1600	1760
1941	2640	2760	2890	2780	2980	2830	2210	2000	2430	2310	2430	2540	2300	2490	2320	2320
Kilograms of water per kilogram of crops (dry weight)																
1939*	383	360	422	410	.....	.....	625	456	359	349	389	378	.....	.....	501	375
1939†	266	255	290	295	.....	.....	388	277	246	242	276	263	.....	.....	320	254
1940*	406	423	443	433	600	515	832	529	493	565	480	530	673	495	915	548
1940†	242	251	262	246	335	301	432	286	274	325	267	292	354	277	457	298
1941*	234	268	263	255	300	273	305	232	304	346	302	331	351	368	352	343
1941†	162	190	190	182	220	196	195	140	204	226	202	227	229	249	231	224
Kilograms of water per kilogram of crops plus roots																
1939*	304	297	315	314	.....	.....	422	338	289	283	301	304	.....	.....	359	295
1939†	211	210	216	227	.....	.....	262	220	198	196	214	212	.....	.....	230	200
1940–41* ...	242	262	261	255	297	283	320	256	286	329	282	311	325	321	328	317
1940–41† ...	200	218	218	211	246	235	254	201	232	266	228	253	259	262	270	254

* Calculated from the total water transpired.

† Calculated from the total water transpired less 800 kg., to correct for evaporation from surface of sand and give a minimum value for true transpiration.

TABLE IX

TOTAL SULPHUR IN THE TOPS AND ROOTS OF 1939 ALFALFA

Crop	High pH						Low pH					
	High S		Medium S		Low S		High S		Medium S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
	%	%	%	%	%	%	%	%	%	%	%	%
1st	0.25	0.32	0.21	0.30	0.17	0.27	0.26	0.32	0.24	0.30	0.16	0.23
2nd	0.27	0.35	0.23	0.32	0.12	0.19	0.28	0.33	0.23	0.30	0.11	0.19
3rd	0.31	0.40	0.27	0.37	0.14	0.19	0.33	0.39	0.33	0.38	0.12	0.24
4th	0.29	0.45	0.25	0.45	0.12	0.25	0.33	0.43	0.32	0.43	0.11	0.27
Weighted mean of crops	0.28	0.37	0.24	0.35	0.13	0.22	0.30	0.36	0.28	0.35	0.12	0.23
Weighted mean of leaves	0.60	0.85	0.62	0.86	0.26	0.53	0.60	0.88	0.53	0.86	0.26	0.49
Crowns	0.22	0.21	0.14	0.15	0.10	0.11	0.21	0.21	0.17	0.18	0.10	0.13
Roots	0.20	0.18	0.13	0.14	0.05	0.08	0.20	0.23	0.14	0.17	0.06	0.09
Hair roots	0.58	0.61	0.45	0.49	0.34	0.50	0.83	0.95	0.78	0.76	0.37	0.46
Weighted mean tops plus roots	0.28	0.35	0.23	0.32	0.13	0.20	0.30	0.36	0.28	0.34	0.13	0.22

parable high nutrient data for 1940–1941, indicating that the amount of water used in producing unit amount of total dry matter, including roots, was not greatly different in the first and second years of growth. As indicated later, this difference was eliminated completely when the water losses from the surface of the sand were considered.

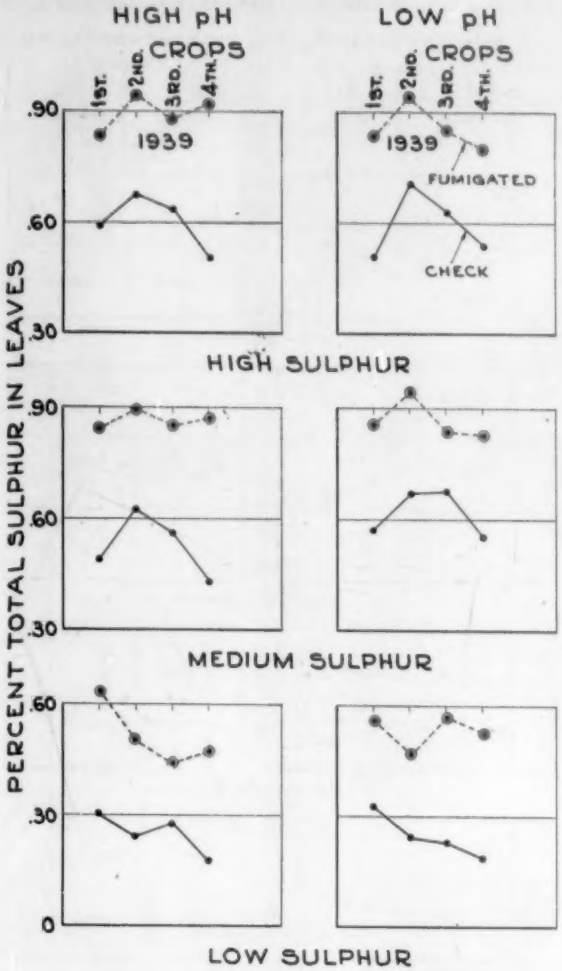


FIG. 4. Sulphur content of the alfalfa leaves at the harvest of each crop in 1939.

Water transpired per unit of dry matter was appreciably higher at the low nutrient level than at the high, owing to the smaller crops on the former plots. Particularly large amounts of water were used by most of the check plots at low and medium-low sulphur levels, as compared with the fumigated plots. With medium and high sulphur there was no appreciable fumigation effect.

When allowance was made for the evaporation of water from the surface of the sand, the foregoing relationships were not materially changed

though some of the differences between the high and low nutrient levels disappeared, and the comparable high nutrient 1939 and 1940–1941 crops plus roots data became nearly identical. It should be noted that the allowance employed (800 kilograms per year) may have over-corrected for these losses, but a smaller allowance would merely have yielded larger transpiration values without affecting the other conclusions. Detailed data

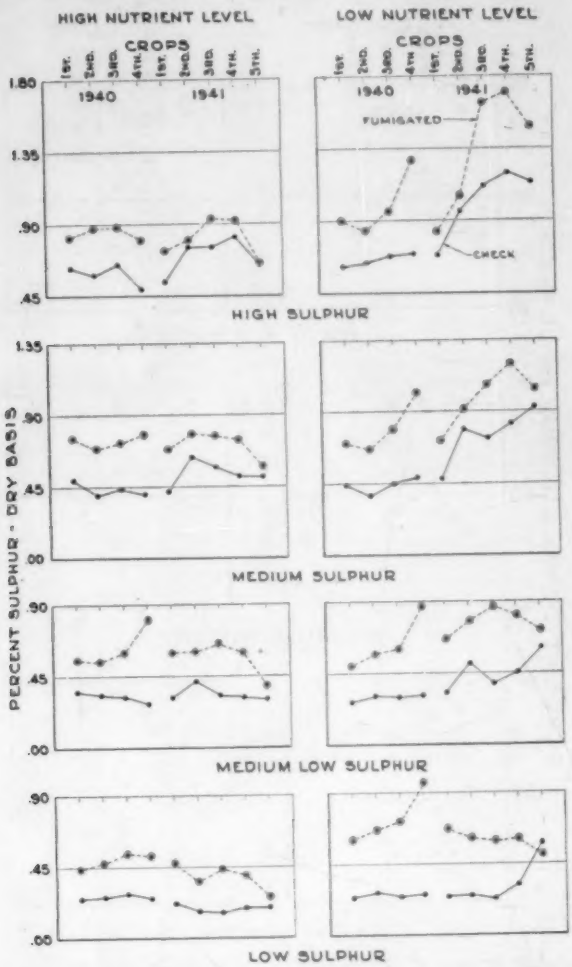


FIG. 5. Sulphur content of the alfalfa leaves at the harvest of each crop in 1940 and 1941.

for the different crops (not shown in table VIII) indicate that the water utilized in the summer was a great deal more than in the spring or autumn. For example, the value for an average first crop in 1941 was about 150 and for the corresponding fourth crop about 400 kilograms per kilogram of dry matter. This large divergence still remained after making allowance for evaporation from the surface of the sand.

TABLE X

TOTAL SULPHUR IN THE TOPS AND ROOTS OF 1940–1941 ALFALFA

Crop	High nutrient level								Low nutrient level							
	High S		Medium S		Medium-low S		Low S		High S		Medium S		Medium-low S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
							1940									
1st	0.26	0.40	0.22	0.31	0.15	0.23	0.13	0.24	0.39	0.44	0.29	0.38	0.24	0.34	0.16	0.23
2nd	0.27	0.37	0.21	0.29	0.18	0.25	0.15	0.24	0.33	0.41	0.20	0.30	0.20	0.32	0.16	0.29
3rd	0.34	0.41	0.21	0.33	0.17	0.29	0.16	0.25	0.34	0.44	0.24	0.38	0.17	0.29	0.19	0.30
4th	0.35	0.51	0.25	0.52	0.20	0.47	0.19	0.40	0.49	0.82	0.33	0.66	0.23	0.54	0.20	0.50
							1941									
1st	0.24	0.28	0.18	0.28	0.16	0.24	0.10	0.21	0.32	0.39	0.25	0.31	0.18	0.27	0.11	0.27
2nd	0.37	0.38	0.30	0.35	0.21	0.29	0.09	0.17	0.43	0.48	0.35	0.41	0.27	0.38	0.13	0.27
3rd	0.35	0.40	0.24	0.31	0.16	0.25	0.10	0.18	0.48	0.60	0.30	0.39	0.18	0.32	0.12	0.21
4th	0.38	0.42	0.28	0.31	0.15	0.27	0.09	0.16	0.56	0.74	0.39	0.50	0.21	0.33	0.15	0.24
5th	0.40	0.39	0.32	0.31	0.19	0.24	0.12	0.15	0.70	1.10	0.57	0.60	0.45	0.48	0.37	0.32
Crowns	0.22	0.23	0.15	0.16	0.12	0.15	0.08	0.11	0.25	0.27	0.20	0.20	0.18	0.17	0.14	0.11
Roots	0.22	0.24	0.13	0.13	0.08	0.10	0.06	0.07	0.23	0.24	0.17	0.18	0.11	0.12	0.09	0.10
Hair roots	0.73	0.63	0.49	0.53	0.30	0.36	0.22	0.26	0.61	0.66	0.68	0.74	0.47	0.48	0.56	0.32
Weighted means 1940–1941 crops plus roots	0.33	0.39	0.24	0.31	0.16	0.26	0.11	0.20	0.41	0.52	0.31	0.40	0.21	0.31	0.17	0.26

THE SULPHUR BALANCE SHEET

In order to establish definitely the minimum sulphate concentrations in the nutrient solution necessary for adequate sulphur nutrition and also to determine the amount of sulphur dioxide absorbed in these experiments, it is desirable to make a comparison of the sulphur removed from the nutrient solutions with the amount found in the crops. This comparison should also furnish a criterion for evaluating the operation of the sand culture equipment.

The vegetation has been analyzed for sulphur by the Parr bomb. This simple method gives excellent results if the samples are allowed to stand for at least 6 days after precipitation before filtration. Tables IX and X summarize the total sulphur values for all the crops, including the roots. Figures 3 and 4 give the sulphur content of the leaves of all the crops. The data show an increasing amount of sulphur in the crops with increasing sulphur in the nutrient solutions. The data also indicate an increase due to fumigation with sulphur dioxide. Absorption of sulphur was apparently not influenced by the range of pH employed (table IX). There was a higher concentration of sulphur in the plant tissue at the low nutrient than at the high nutrient level (table X), and particularly in the leaves, as shown in figure 5. This difference was not caused by a difference in rate of absorption of sulphate but rather was due to the fact that the low nutrient plants were smaller than the comparable high nutrient plants. The high sulphur content of the hair roots is noteworthy. These values were about equal to the sulphur content of the leaves. In a majority of cases the check leaves had less sulphur than the hair roots, though the reverse was nearly always true of the fumigated leaves. The hair roots of the fumigated plants generally had more sulphur than those of the check plants. An important exception was the low sulphur-low nutrient pair, in which the high sulphur content of the hair roots of the check plot was associated with improved growth of the fourth and fifth crops as pointed out earlier.

The balance sheet of sulphur absorbed from the nutrient solutions and sulphur found in the 1939 and 1940–1941 crops, including the roots, is given in table XI. Check and fumigated plots are considered side by side. Decreasing sulphur additions are shown in the columns. The data show clearly that if sulphur was available either in solution as sulphate or as sulphur dioxide, it was readily absorbed by the plants. For an unknown reason, more sulphur was found in the plants of the check plots than was removed from the nutrient solutions. Evidently the check plants received sulphur from a source other than the nutrient solution. This sulphur is listed in the table as "unaccounted for."

It was presumed that the principal source of this sulphur was the tar lining the tanks, which contained about 3 to 4 per cent. sulphur, though long contact of distilled water with this tar has failed to dissolve more than a trace of sulphate. Since, however, the hair roots of the alfalfa were found to be thickly distributed over the tar surface, adhering firmly but

TABLE XI

BALANCE SHEET OF SULPHUR ABSORBED FROM THE NUTRIENT SOLUTIONS AND SULPHUR FOUND IN THE PLANTS

YEAR	TREATMENT	SULPHUR IN					
		CHECK PLOTS			FUMIGATED PLOTS		
		FROM SOLUTION	IN PLANTS	UNACCOUNTED FOR	FROM SOLUTION	FROM SO_2 (CALCULATED)	IN PLANTS
		grams S per plot					
1939	High pH — High S	22.8	24.3	1.5	19.0	12.0	32.5
	High pH — Medium S	16.9	18.9	2.0	12.8	11.0	25.8
	High pH — Low S	3.3	6.6	3.3	2.4	7.9	13.6
1939	Low pH — High S	25.0	26.8	1.8	23.2	8.3	33.3
	Low pH — Medium S	22.3	25.7	3.4	18.3	7.3	29.0
	Low pH — Low S	3.6	7.9	4.3	3.4	10.7	18.4
1940–1941	High nutrient — High S	56.6	62.7	6.1	46.3	14.2	66.3
	High nutrient — Medium S	29.5	42.9	13.4	24.8	17.0	55.2
	High nutrient — Medium-Low S	17.4	26.3	8.9	11.0	22.7	42.6
	High nutrient — Low S	1.5	12.9	11.4	1.1	14.9	27.4
1940–1941	Low nutrient — High S	53.3	61.5	8.2	40.3	19.2	67.7
	Low nutrient — Medium S	31.0	47.8	16.8	17.0	21.9	55.7
	Low nutrient — Medium-Low S	11.2	26.8	15.5	7.8	19.7	43.0
	Low nutrient — Low S	2.4	20.3	17.8	1.7	14.9	34.4

not penetrating the layer, the possibility remained that the roots could have acquired sulphur directly from the tar in spite of its low solubility. For this reason, before the 1942 season started, the sand boxes were lined with plate glass, mounted in the tar. Only a few narrow seams of tar were left exposed, thus reducing the uncovered tar surface to a negligible area.

In spite of this extraneous source of sulphur, the balance sheet gives an excellent picture of the sulphur exchange. In every case the fumigated plots showed less absorption from the nutrient solution than the corresponding check plots. Evidently the additional supply through the leaves reduced the intake through the roots. The table gives an estimate of the amount of sulphur absorbed as sulphur dioxide on the assumption that the "unaccounted for" sulphur was the same in each fumigated plot as in its check. The amount of sulphur thus ascribed to sulphur dioxide was approximately the same in the different plots of each group, as would be expected from the fact that the fumigations were applied uniformly to all the treated plots. The "unaccounted for" sulphur was somewhat smaller in the high sulphur plots than with the other treatments, suggesting that this supply was not drawn on so heavily when there was adequate sulphate in solution.

Consideration of the values of the "unaccounted for" sulphur indicates that the low-sulphur check plots of 1940–1941 received a total amount of sulphur approximately equal to the amount accounted for as removed from the medium-low sulphur solutions. It is, therefore, probable that if the nutrient solution had been the sole source of the sulphur, the growth obtained on the low-sulphur plots could have been obtained with a solution about 0.8–1.0 p.p.m. sulphur, and the growth on the medium-low sulphur and medium sulphur, with concentrations 0.5–0.8 p.p.m. greater than those employed. Sulphur deficiency would then be expected with less than about 1.5 to 2.0 p.p.m. sulphate sulphur in the nutrient solution at the growth rates of these experiments.

Summary

Alfalfa has been grown in the large sand-culture equipment for three years. A wide range of concentrations of sulphate was employed in the nutrient solution, and one-half the plots were fumigated with 0.1 p.p.m. sulphur dioxide for about 7 hours each day and 6 days a week throughout nearly the whole life of each crop.

In 1939, 6 pairs of plots were studied using a nutrient solution in which the concentration of the principal nutrients was about one-half that of HOAGLAND's solution. Seedlings were started in the greenhouse and 4 large crops, including finally the roots, were harvested during the year. Three pairs of these plots were maintained at about pH 5.5; the others at about pH 7.0. Two pairs of nutrient solutions each had nominal sulphate concentrations corresponding to 0, 5, and 90 p.p.m. sulphur. The "zero" solution actually contained about 0.5 p.p.m.

In 1940 new seedlings were started for 8 pairs of plots. Four crops were harvested in 1940 and 5 crops, in addition to the roots, in 1941. A pH of about 5.5 was maintained throughout. Four pairs of plots had a nutrient solution of about 0.5 HOAGLAND'S concentration; the other 4 pairs after starting at this strength, were progressively diluted until they reached 3 per cent. of the initial concentration. Two pairs of the nutrient solutions each had nominal sulphate concentrations of 0 (actually 0.2–0.3), 0.8, 1.5, and 10 p.p.m. sulphur.

The results of the experiments were as follows:

1. A pH of 5.5 was more favorable for growth of alfalfa than a pH of 7.0, with the lower sulphate concentrations. There was no pH effect with 90 p.p.m. sulphate sulphur in the nutrient solution.

2. The leaves of the sulphur-deficient plants were definitely chlorotic.

3. Yield of the sulphur-deficient plots was appreciably less than the yield of the plots with adequate sulphur.

4. Yield of the sulphur-deficient plots was improved by fumigation with sulphur dioxide. This source of sulphur was less efficient than sulphate for purposes of nutrition, at the concentrations applied.

5. Yield of the fumigated plots which had adequate amounts of sulphate in the nutrient solution was statistically the same as the yield of the unfumigated checks.

6. The root systems of the plots did not vary as greatly in weight as did the crops. In 1941, the check roots were heavier than the corresponding fumigated roots in seven out of eight pairs.

7. Net assimilation data from carbon dioxide exchange measurements confirmed the yield data.

8. Transpiration values, calculated from the water losses from the supply tanks, fell within a rather narrow range in each experiment, except that the low sulphur plots gave appreciably lower results. There was no discernible effect due to fumigation in the plots with adequate sulphur. In 1939 and 1940 the low and medium-low fumigated plots transpired only 5 to 12 per cent. more water than their checks, though they had 25 to 84 per cent. more top growth.

9. The water transpired per unit of top growth was greater in first year 1939 and 1940 crops than in the second year 1941 crops, but the amount per unit of total growth including roots was about the same in all three years. Transpiration per unit of top growth was particularly high in the sulphur-deficient check plots. The sulphur dioxide fumigations had no appreciable effect on transpiration in the plots adequately supplied with sulphate sulphur. Summer crops transpired much more water than the spring and autumn crops. These relationships were not greatly changed when the transpiration values per unit of dry matter were corrected for water evaporated from the surface of the sand.

10. Sulphur analyses of the vegetation showed that absorption of this element was increased by increasing the sulphate concentration of the nu-

trient solution or by sulphur dioxide fumigation. Absorption was not influenced by the range of pH considered. Nearly the same amount of absorption of sulphur occurred in comparable plots at the low and high nutrient levels, but owing to slower growth of the low nutrient plants, the sulphur concentrations in them were greater than in the high nutrient plants.

11. The sulphur balance sheet indicated that the plants received some sulphur from a source other than the nutrient solution. The amount of the unaccounted-for sulphur was least in the high sulphur plots.

12. The fumigated plants absorbed less sulphur from the nutrient solution than did the check plants.

13. With less than 1 p.p.m. sulphate sulphur in the nutrient solution there was definite evidence of sulphur deficiency in the plants. Taking the "unaccounted for" sulphur into consideration, it appeared that deficiency symptoms could be expected under the conditions of these experiments with less than about 1.5 to 2.0 p.p.m. sulphate sulphur, if the nutrient solution were the only source of the sulphur.

Acknowledgment is made to Mr. LYNN BROWN for assistance with the experimental work, and to Messrs. RODNEY WALKER and MILTON R. BERNTSON for assistance with the data and records.

DEPARTMENT OF AGRICULTURAL RESEARCH
AMERICAN SMELTING AND REFINING COMPANY
SALT LAKE CITY, UTAH

LITERATURE CITED

1. ALWAY, F. J. A nutrient element slighted in agricultural research. Jour. Amer. Soc. Agron. **32**: 913–921. 1940.
2. ————————, MARCH, A. W., and METHLEY, W. J. Sufficiency of atmospheric sulfur for maximum crop yields. Proc. Soil Sci. Soc. Amer. **2**: 229–238. 1937.
3. HART, E. B., and PETERSON, W. H. Sulphur requirements of farm crops in relation to the soil and air supply. Wisconsin Agr. Exp. Sta. Research Bull. 14. pp. 21. 1911.
4. HOAGLAND, D. R. Optimum nutrient solutions for plants. Science n.s. **52**: 562–564. 1920.
5. KATZ, MORRIS, and LEDINGHAM, G. A. Effect of sulphur dioxide on vegetation. Chapter XIII. Experiments on yield of barley and alfalfa. National Research Council of Canada. N.R.C. no. 815. pp. 447. 1939.
6. POWERS, W. L. Sulphur in relation to soil fertility. Oregon Agr. Exp. Sta. Bull. 199. 1923.
7. SCHERTZ, F. M. The extraction and separation of chlorophyll ($\alpha + \beta$) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.

8. SETTERSTROM, CARL, ZIMMERMAN, P. W., and CROCKER, WILLIAM. Effect of low concentrations of sulphur dioxide on yield of alfalfa and Cruciferae. Contrib. Boyce Thompson Inst. **9**: 179–198. 1938.
9. THOMAS, MOYER D., HENDRICKS, RUSSEL H., IVIE, JAMES O., and HILL, GEO. R. An installation of large sand-culture beds surmounted by individual air-conditioned greenhouses. Plant Physiol. **18**: 334–344. 1943.
10. ————, and HILL, GEO. R. Relation of sulphur dioxide in the atmosphere to photosynthesis and respiration of alfalfa. Plant Physiol. **12**: 309–383. 1937.
11. ————, and ————. The continuous measurement of photosynthesis, respiration, and transpiration of alfalfa and wheat growing under field conditions. Plant Physiol. **12**: 285–307. 1937.

POLARIZATION AND STIMULATION OF THE ONION ROOT BY DIRECT CURRENT

L. Joe Berry and Rosalie C. Hoyt

(WITH TEN FIGURES)

Introduction

Previous work has shown (**1**) that the passage of a constant, positive electric current through an onion root (a) increased the positivity of the observed[1] electric potential when the current was sent up the root (polarization) and (b) decreased the observed potential when the current was sent down the root (depolarization). This confirmed earlier observations by Marsh (**11**) and was in agreement with similar experiments by Wilks (**19**) and Clark (**8**) with the Avena coleoptile, Rosene (**17**) with the Douglas fir, and Rehm (**15**) with *Phaseolus multiflorus*. This effect was due to changes within the root and not at the contacts. The magnitude of the change was greater when the potential was decreased than when it was increased. Moreover, basal segments of the root exhibited smaller responses to a given current flow than apical segments. Both hydrogen cyanide and hydrogen reduced the inherent electric potential to essentially zero and at the same time produced little, if any, change in the action of applied current. These effects were reversible. The minimum current required for producing (a) or (b) was well within the range of current magnitude that could be maintained by the root in an external circuit. It was higher in basal segments than in apical segments. With constant current applied for increasing time periods, the effect reached a maximum. These results were interpreted as being due to an ionic accumulation at "phase boundaries" within the cell. This potential due to polarization was shown to be independent of inherent potential. Several "anomalous" curves (**1**, fig. 9) were obtained, however, in which there was a decrease with upward current in contrast to (a) above, and it was thought that the current acted as a stimulus for the root with a resultant negativity in inherent P.D. similar to an effect produced by Marsh (**12**) mechanically in the onion root. Rehm (**15**) also found such a change but only when the applied current was several fold larger than that required for polarization. In a series of studies on the effect of applied direct current on the potentials of the coenocytic algae, Blinks (**4, 5, 6**) found that currents opposing the inherent flow increased the P.D. while series currents decreased the P.D. He was able, however, to show that with increased opposing current a stimulated condition could be produced in which the potential decreased rather than increased. The ease with which this effect could be obtained was subject to the previous history of the cells, particularly the ionic constituents of the medium in which they were kept.

[1] The usual convention is to measure the electric potential of the distal contact with respect to the earthed proximal contact.

In the present report, data will be given which will throw additional light on the changes produced in the polarity potential of the root of *Allium cepa* by small applied currents and which confirm earlier observations. It is believed that they show: (a), an increased magnitude of applied current may stimulate[2] rather than polarize the root and when this occurs the effect is reversible; (b), the apical segment of the root is more readily stimulated than more basal segments of the root; (c), the size of the response to a given amount of stimulating current is reversibly dependent on the time of application of current and may even appear as a polarization if time is sufficiently long; (d), the magnitude of current required to stimulate rather than polarize a root varies from root to root and seems to be less at lower temperatures; (e), a stimulated root is still polarizable; (f), a root whose inherent potential is lowered by the removal of oxygen can no longer be stimulated by a current which stimulated the root in air but is polarized by the same current; (g), there is a quantitative difference between polarization and depolarization and also a difference in magnitude of these two types of response in apical and basal segments; (h), the effects of current flow are relatively independent of the age and length of the root; and (i), the "electrodes" within the root responsible for polarization and for stimulation are to a large degree different and independent of one another.

Method

The apparatus used for this investigation was patterned after that described in earlier papers (**1, 2**) in which the arrangement of the electrodes and contacts within the experimental chamber and the electrical circuit and switching diagram are described. The potential measuring instrument was an RCA Ultrasensitive DC Meter with a sensitivity of two millivolts per scale division and could be read to an accuracy of one-fourth division. The period of the instrument was three seconds and its stability was excellent. Current was measured with a Leeds and Northrop type 2420C galvanometer calibrated so that one scale division equalled 0.10 microampere.

Roots were grown as usual in aerated tap water in an aquarium with the onions supported in flanged glass tubes which were held in paraffined wooden slats. All roots but one were trimmed from the bulb before it was transferred to the experimental chamber. The length of individual roots used in different experiments varied from 30 to 55 mm. and their diameter from 0.53 to 0.72 mm. Contacts were moved into place around the root and a thirty-minute period of equilibration was allowed before beginning the ex-

[2] For convenience of terminology, *polarization* will be used throughout this paper to signify an increase in positivity of the observed potential when current is sent up the root; *depolarization* will signify a decrease in positivity of potential when current is sent down the root; and *stimulation* will signify a change in potential contrary to these. These definitions are in good usage as long as the distal contact is positive to the proximal contact but become contradictory when the inherent P.D. is reversed. It is felt that the inaccuracies resulting from these arbitrary definitions will be less confusing to the reader than strict adherence to the usual meaning of the words.

periment. Growth was recorded at thirty-minute intervals throughout the duration of an experiment with the aid of an ocular micrometer in a horizontal microscope. The root was always returned to the aquarium at the end of a test and its growth again measured the following day. Failure to grow was taken as evidence of injury and three experiments were discarded for this reason. Electrodes of zinc-amalgam zinc sulphate were used. Previous work using separate contacts for applying current (1) has shown that the effect of current flow is on the root segment between the contacts and not at the contacts themselves. Therefore, in the experiments reported here the same contacts were employed both for applying current and measuring potential. In this way the extreme tip of the root could be included in the P.D. measurements. The electrodes were checked at the beginning and end of each run to assure their isoelectric and non-polarizable condition. An upper glass ring and lower glass cup were used as contacts on the root and contained the tap water medium in which the roots were grown.

To change temperature during an experiment, water in a bath was circulated by means of a small centrifugal pump through a double walled copper experimental chamber. The front and back of this chamber had glass windows and the outer wall was heavily paraffined to reduce heat exchange with the surroundings. The water was cooled with ice and the temperature was regulated manually to within 1° C. A thermometer extending into the chamber with the bulb just above the onion was used to follow the temperature. Room temperature varied between 18° and 29° C. during the course of the investigation. This range proved to be of great value in revealing the importance of temperature in determining the type of response elicited by current; *i.e.*, polarization or stimulation. Control experiments show that the electrodes undergo a considerable change in conductivity but remain isoelectric over the temperature range used.

Cylinders of commercial oxygen and hydrogen were used as a source of these gases. The gases were saturated with water vapor by sending them through gas washing towers before passing them into the experimental chamber.

The strength, duration, and direction of current flow through the root was varied from experiment to experiment and at times within a given experiment. During the application of current, no potential measurement was made but the switch from the root to the Sensitive Meter was turned to the "on" position at the same time the current was turned off. A reading was made within three seconds and at 15-second intervals for the first minute thereafter. Subsequent readings were made at 30-second intervals. Some points were omitted in redrawing the curves for publication if no change in the shape of the curve resulted.

Since the pathways of current flow through a root are not known, the current density may not be uniform over a given cross-sectional area and may vary along the root axis. For this reason, it is felt that current densities have an unknown significance and current magnitudes are therefore given only in microamperes.

Procedures and results

Effect of current of variable magnitude and constant duration on the electric potential when sent alternately up and down the root

With contacts placed at 0 and 5 millimeters above the root tip, current of varying strength was sent alternately up and then down the root for 30

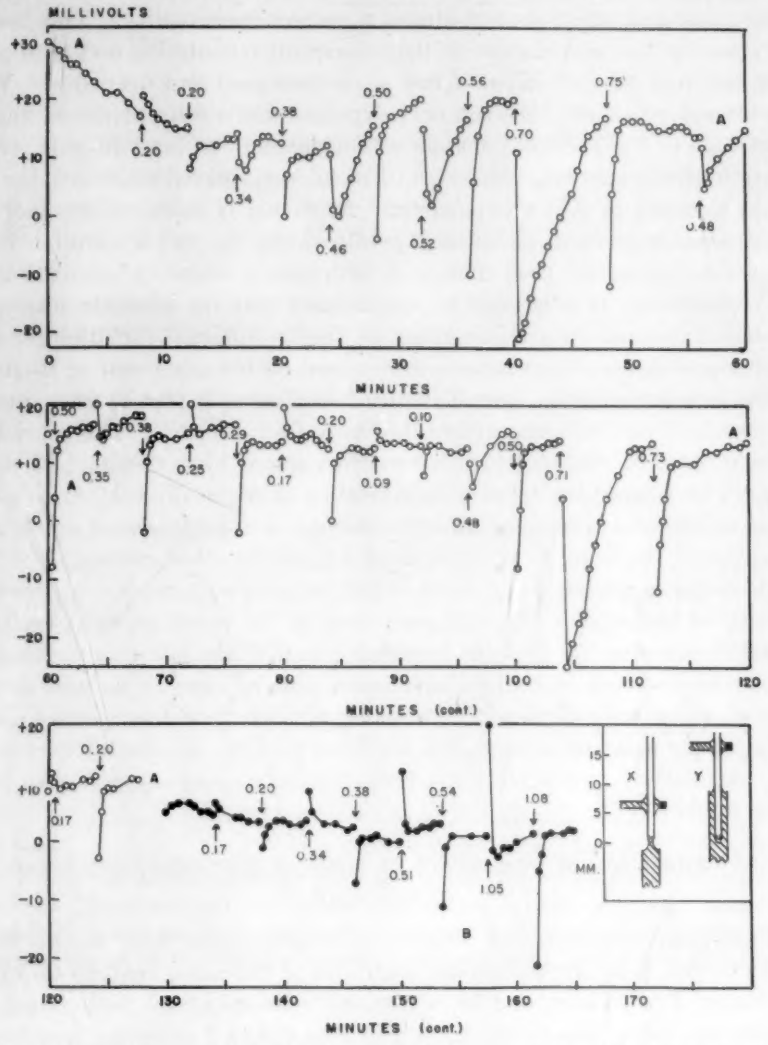


FIG. 1. The effect of applied current of variable magnitude on the inherent potential of the onion root. Arrows pointing upward indicate positive current sent up the root, arrows pointing downward indicate positive current sent down the root. The number with each arrow indicates the magnitude of the applied current in microamperes. In curve A the contacts were at 0 and 5 mm. and in curve B they were at 5 and 10 mm. on the same root, as shown in insets X and Y. The time of current flow was 30 seconds.

seconds and the change in potential was followed. The periods between the application of current were four minutes for the lower magnitudes and eight minutes for the higher magnitudes. Curve A, figure 1, is typical of the results obtained. Inset X, figure 1, shows the position of the contacts on the root. Upward current when sufficiently small (0.20 microampere) polarized the root but as the magnitude was increased in small increments to 0.70 microampere, it stimulated the root. With a reduction in current, a return to the polarizing effect showed almost a perfect reversibility. This reversibility was further emphasized by the subsequent stimulating and then polarizing action of the current as it was again increased and decreased. When current was sent down the root, only depolarizations were produced and the magnitude of the potential change was dependent, at least in part, on the strength of the current. However, 0.56 microampere depolarized the root almost as much as 0.75 microampere. Attention is called to the fact that the decrease in inherent potential is greater when the root is stimulated than it is when depolarized even though in both cases a negative potential is initially observed. It must also be emphasized that the absolute magnitude of current necessary for stimulating the root is different for different roots and temperatures. The contacts were raised on the same root to 10 and 15 millimeters above the tip, inset Y, figure 1, and curve B (fig. 1) was obtained. All time intervals between current flow were four minutes. This more basal segment was not stimulated with upward current even though 1.05 microamperes were used; but there is an indication of slight stimulation, as shown by the small downward cusp, when the maximum current passed up the root. This type of response, however, is more evident in other curves.

A comparison of the magnitude of polarization with depolarization when currents of comparable strength were used in the apical segment verify an earlier observation (1) that the former is less than the latter even though in the earlier observation the absolute magnitude of current as well as time of application were different. The same relation to a less marked degree holds for the basal segment. It is also seen that the amount of depolarization, particularly, is less for a given current in a basal segment than in an apical segment (1).

A COMPARISON OF THE EFFECT OF CURRENT ON VARIOUS SEGMENTS OF THE ROOT

To further emphasize the differential behavior of a polar system to the same current, four five-millimeter segments of the same root (0 to 5 mm., 3 to 8 mm., 5 to 10 mm., and 10 to 15 mm.) were subjected to the same current successively. Insets W, X, Y, and Z in figure 2 show the positions of the contacts on the root that gave curves A, B, C, and D, respectively. Curve A′ was obtained by returning the contacts to position W. The current was sent alternately up and then down the root with eight minutes between each application. With this particular root, 2.00 microamperes for 30 seconds were required for a stimulating current (curves A and A′) but

only the first five millimeters of the root showed this type of response while the second segment gave some indication of being only partially stimulated (curve B). The two more basal segments, like the one in figure 1, were only polarized and depolarized (curves C and D).

The depolarization was progressively less as more basal segments were used if curve A′ is taken as typical for the apex. The failure of curve A

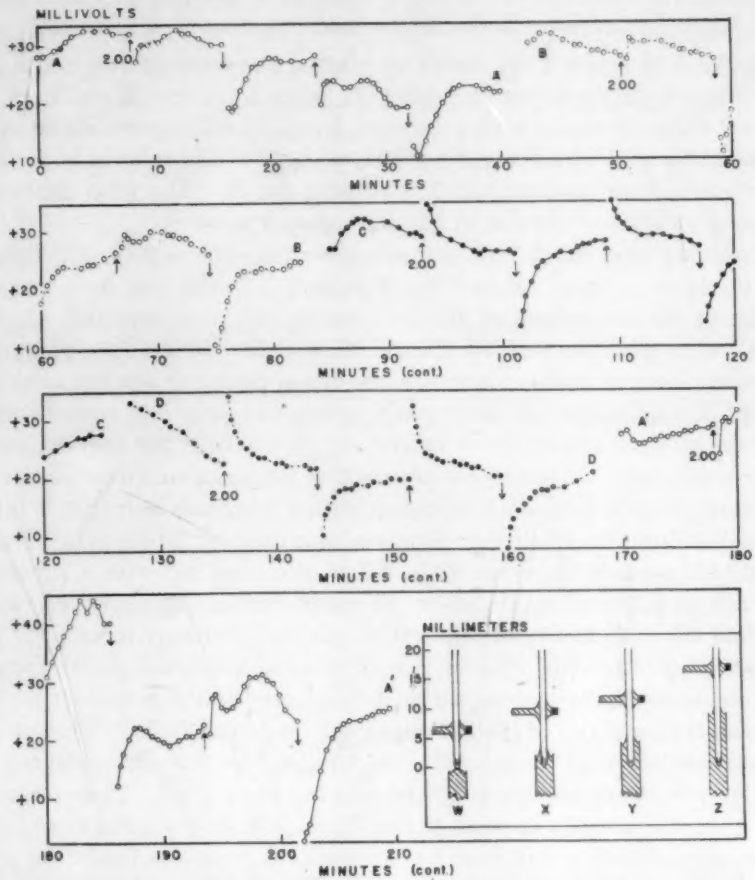


FIG. 2. A comparison of the effect of a stimulating current on various segments of the root. The direction of the arrows indicates the direction of applied current. The magnitude of the current was 2.00 microamperes and the duration of flow was 30 seconds. The positions of the contacts on the same root were: curve A, 0 and 5 mm.; curve B, 3 and 8 mm.; curve C, 5 and 10 mm.; curve D, 10 and 15 mm.; curve A′, 0 and 5 mm. (insets W, X, Y, Z).

to show this characteristic suggests that a partial stimulation occurred with downward current but its effect was masked by the depolarization imposed on it. This of course assumes that when the contacts were returned to position W, the proximal cells of the segment were less easily stimulated than originally and therefore the depolarizations in curve A′ were greater than

those in curve A. Evidence for such a suggested change in irritability is to be found in the work with alternating current (**3**). Polarizations were again less than depolarizations except in D, where they were essentially equal. As shown in curve 9, this seems to be somewhat characteristic of more basal segments.

Effect of current of constant magnitude and variable time on the observed potential of different segments of the root

Curve A of figure 3 was found by placing the contacts at 0 and 5 millimeters above the tip of the root, inset X, figure 3. Curve B and inset Y of figure 3 show the result with a segment 5 and 10 millimeters above the tip of a second root. The time scale refers to the absolute value only for curve A with zero time beginning at 120 minutes for B. The time interval between application of current in all curves was 8 minutes.

Opposing current of 0.40 microampere was sufficient to stimulate the root of curve A when applied for 5 seconds. There was no appreciable change in the magnitude of the decrease in inherent potential when the periods of application were 10, 15, and 30 seconds. When current was sent up the root for one minute, however, the initial potential was the same after current flow as before but there was a subsequent drop and recovery which gave evidence of a stimulated condition. Increasing the current to 1.00 microampere with the same root reveals that the maximum decrease in P.D. as a result of stimulation was obtained within 5 seconds and that it became less as the duration of current flow was increased to 10 seconds. For 30, 60, and 120 seconds there was an initial polarization but with a subsequent total fall in potential much below the initial value. Recovery was almost complete after the 30-second application but was obviously incomplete after the two longest periods. There is a reversal of electrical polarity during this time and current sent up the root *decreases* the P.D. to about zero but increases the positivity of the apical contact (see footnote 2). This root was still capable of being stimulated even though the inherent potential was negative when current passed up the root for 15 seconds. Time periods as short as it was possible to work the switches (not shown on curves), failed to give any indication that there was an initial polarization preceding stimulation similar to that found by Blinks (**5, 6**). In his technique it was possible to apply current and make potential readings simultaneously. It is apparent from this curve that a stimulated region is still polarizable and that the observed potential change following applied current is an apparent algebraic summation of two oppositely oriented changes induced in the system. The response to stimulation must be the more rapid change of the two since it is the first to appear.

Since Berry (**1**) showed[3] the magnitude of polarization for a given cur-

[3] 0.50 microampere was the only current used in the earlier study except in determining the threshold of polarization. Similarly, the duration of current flow was kept at 60 seconds except for the investigation of the magnitude of polarization as affected by time. The choice of these two values was dictated by the results of Marsh (**11**) in which he found these values to be without injury and to render decisive results.

rent increased with time up to about one minute then, in this case, as the duration of current flow increased the change in potential due to polarization became greater and the observed effect of stimulation diminished. How-

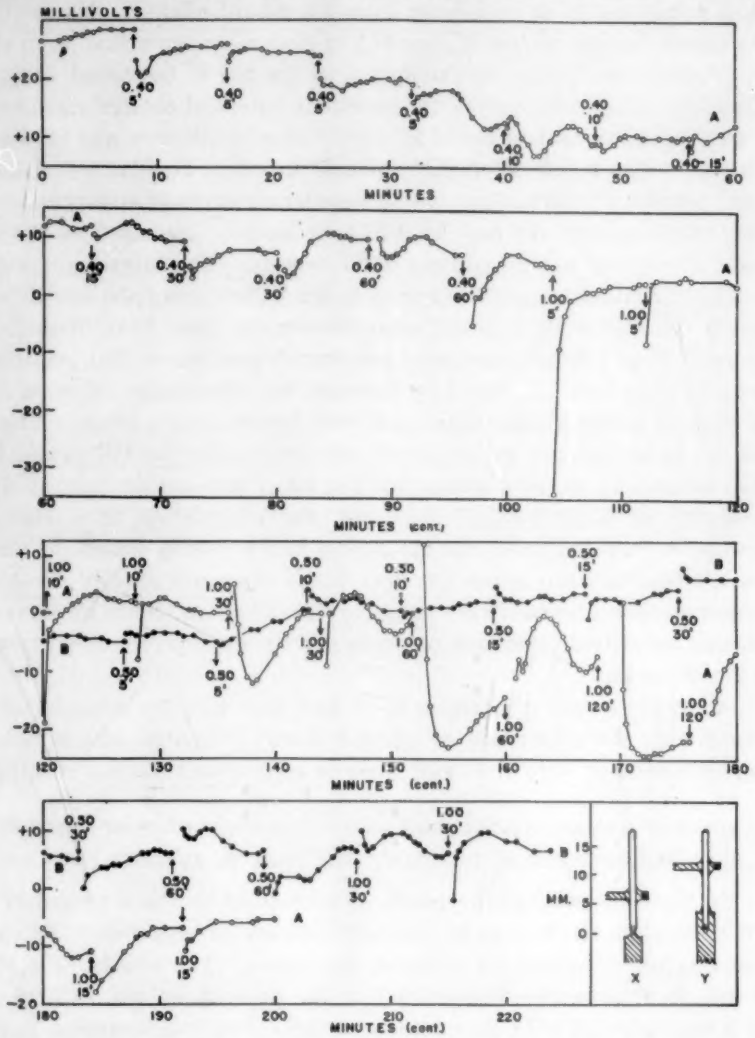


FIG. 3. The effect on inherent potential of current applied for varying lengths of time. The direction of the arrows indicates the direction of the applied current. The numbers with each arrow indicate the magnitude of current in microamperes, and the duration of current flow in seconds (′). The positions of the contacts were: curve A, 0 and 5 mm. (inset X); curve B, 5 and 10 mm. (inset Y). Curves A and B were obtained with different roots.

ever, these results may also be attributed in part to some type of change in the cells along the root axis in which the response to stimulation decreases with time of current flow. Such an effect has been found with AC. (**3**).

The similarity between the changes produced in curve A when 1.00 microampere was sent up the root for 60 and for 120 seconds, indicates that for a given set of conditions the effects due to stimulation and to polarization reach a maximum value and hence give a constant change. No doubt one of the causes for the earlier failure (1) to find more roots that were stimulated ("anomalies") may be attributed to the use of 60-second periods of application. In several curves, the resultant potential change after current flow was similar to that in curve B after 1.00 microampere was applied for 30 seconds. The behavior of the potential in curve B, figure 1, following upward current of 1.05 microamperes, mentioned above, is apparent.

Depolarizations of the root by 0.40 microampere increased as the duration of current flow was lengthened to 60 seconds. The shorter applications show some variations of small magnitude but differences from one depolarization to the next with constant conditions make these have doubtful significance. With 1.00 microampere, however, it was found that, contrary to the results when current passed up the root, the stimulating effect of downward current becomes more noticeable with longer time periods. The failure of the 15-second interval of flow to stimulate after the 120-second interval had stimulated the root shows that the effect was not necessarily due to some injury or abnormality. Moreover, the stimulation of a root with downward current suggests that the region in the root in which the current enters (in this case the upper contact) is the one more readily stimulated. This interpretation probably accounts for the failure to obtain an increasing depolarization with 1.00 microampere as the time of current flow increased from 5 to 30 seconds.

Curve B is typical of a segment of root in which no stimulation was apparent with the magnitude of current used. Potential alterations progressively increased with time and were in accord with earlier results (1).

Effect of decreasing the time between applications of current on the response of inherent potential to current flow

In the previous section, the result of increasing the time period of current flow through the root gave results which are interpreted as being due to a summation of oppositely oriented responses. The ability of a stimulated root to polarize is demonstrated in the present section. Curve A of figure 4 was obtained with the contacts at the apical 5 millimeters, inset X. This particular root required 1.50 microamperes for stimulation as indicated by the first part of the curve. The duration of current flow in all cases was 15 seconds. The root was stimulated at 8-minute intervals and after the fourth time the same magnitude of current was sent up the root one and one-half minutes later and again after two minutes. Following an eight minute recovery period, the three applications of current with the short time intervals between were repeated and so on. In the first two cases both the first and the second application of current stimulated the root. The third application gave rise only to a polarization. In the rest of the series,

only the first of the current applications stimulated and the other two polarized the root. Thus, before recovery from stimulation is complete, it is possible to polarize the root with the same current. Failure of the two more

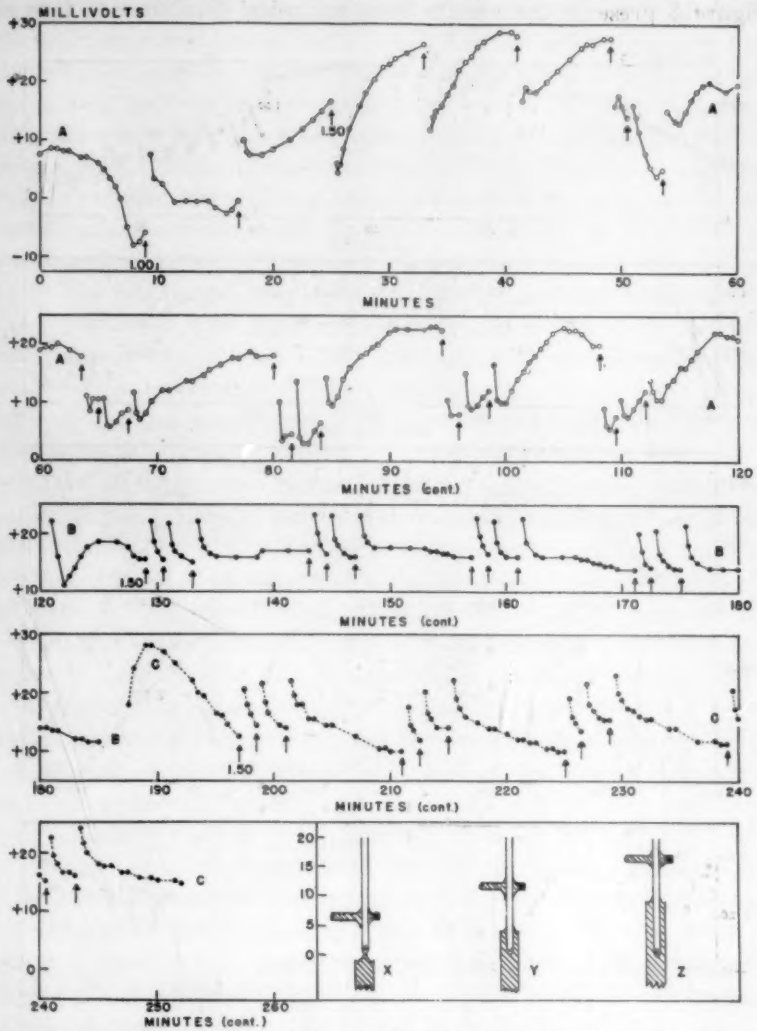


FIG. 4. The effect of decreasing the time between applications of current on the response of inherent potential to current flow. The direction of the arrows indicates the direction of the applied current. The numbers at the arrows give the magnitude of the current in microamperes. (Where no number is given, the current was the same as that last indicated.) The duration of current flow was 15 seconds. Curves A, B, and C were all obtained with the same root and the positions of the contacts were: Curve A, 0 and 5 mm.; curve B, 5 and 10 mm.; curve C, 10 and 15 mm. (insets X, Y, Z).

basal segments (5 and 10 millimeters, and 10 and 15 millimeters up the same root, insets Y and Z) to behave in the same way is shown in curves B and C, respectively (fig. 4). The level of polarization reached in B is

essentially the same for all applications of current. In C, with each successive current flow in a series of three, there is an increase in the absolute potential reached but the magnitude of polarization is approximately equal.

Figure 5 presents the results from an apical 5-millimeter segment of

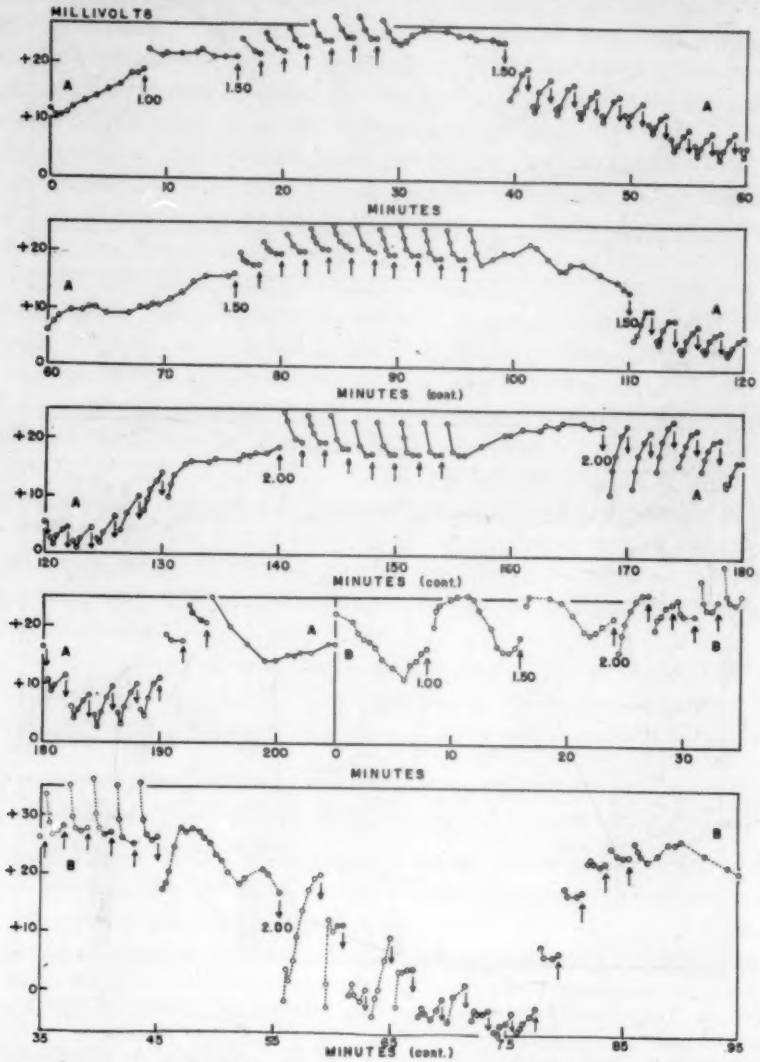


FIG. 5. Same as figure 4. Curves A and B were obtained from two separate roots and the positions of the contacts were: curve A, 0 and 5 mm.; curve B, 0 and 5 mm.

root (contacts placed as shown by inset X, fig. 4) which gave only polarization or depolarization when current was sent either up or down the root every two minutes several times in succession on the time scale. The duration of flow was 30 seconds in each case and curve A is typical of the results obtained. Current of 1.50 microamperes sent up the root seven times be-

tween 16 and 30 minutes and eleven times between 76 and 96 minutes caused a progressively steeper and slightly larger polarization during the first five applications in each series and then produced an essentially constant polarization in the remaining applications. With the magnitude of current raised to 2.00 microamperes between 140 and 155 minutes and applied eight times, all polarizations were essentially alike. This suggests that the charges responsible may be built up gradually with weaker currents to a constant polarization while with stronger currents the initial application achieves a maximum effect. Since it has been shown (**1**) that increasing duration of flow raises polarization to a maximum, it would be expected that increasing the magnitude of current should have a similar effect.

Depolarizing current of 1.50 microamperes was applied eleven times between 40 and 60 minutes and again between 110 and 130 minutes. In the first series not only were smaller depolarizations produced but the type of potential change was altered as the electrical polarity decreased and then became practically constant. In the second series, however, a similar progression of changes was altered in the last three applications by a rather sharp rise in the inherent potential. There is no reason to think that polarizing or depolarizing currents are capable of causing any change in the spontaneous fluctuations in potential that exist in the absence of any known external stimuli (**2**), and hence may alter the *observed* effect of applied current. The series of depolarizations with 2.00 microamperes between 170 and 190 minutes is somewhat similar to the above. It should be noted that at the end of this series polarizing currents were applied three times and the first, as well as the second to a smaller degree, increased the potential without any appreciable fall to a lower value (see also curve B).

Roots capable of being stimulated were treated in a manner similar to the above and curve B, figure 5, is typical of the results obtained. The contacts were placed as shown in X, figure 4. 2.00 microamperes upward current stimulated the root but polarized it after the third application. The same intensity of current sent down the root at the end of this series produced a perfectly normal depolarization. Successive depolarizations produced changes similar to those in curve A but with less regularity in the sequence. Then the final passage of current up the root following the above was again effective in restoring the electrical polarity before normal polarizations were obtained.

The initial fall in potential immediately following the passage of current down the root that sometimes occurs before the rise in potential, appears in several cases in figure 5 (see also figs. 2, curve A; 6; 7; 10). The mechanism involved in this effect of current flow is not understood but the explanation that seems most likely on the basis of our present knowledge is that a small stimulation has been produced and summed with a depolarization. A more rapid recovery from stimulation must occur initially and thereby reduce the potential before recovery from depolarization restores the potential. A similar type of change following the passage of current up the root on the

other hand would depend upon polarization recovery occurring more rapidly than stimulation recovery. However, since AC (**3**) response may also have an initial downward cusp preceding the rise, a differential rate of recovery in apical and basal cells may be the whole basis of such changes in the potential; *i.e.*, in the absence of any but a stimulating action.

Effect of temperature on the "threshold" of stimulation in apical and basal segments of the root

From observations of the effect of room temperature in summer and winter on the "threshold" of stimulation of roots, it was considered desirable to test experimentally the changes produced by temperature on a single root. The technical difficulties involved warrant a brief description. The circulation of cold water through the copper jacket used as a chamber for this part of the work caused a condensation of water vapor on the inner wall. This drying action gave rise to a shrinkage of the root as the temperature was lowered. On raising the temperature, there was a condensation of moisture on the root which lagged behind the chamber in warming. In order to avoid desiccation, the lower cup contact was raised around the root to a height of about 20 to 25 millimeters during the hour in which the temperature was first reduced. No shrinkage was observed during this time but growth was usually less than normal. A perfect equilibrium between water vapor and the air within the chamber was not reached since upon resuming the experiment at the end of this time slight shrinkage was observed. This shrinkage was not more than 0.30 millimeter for the entire period at the low temperature, and with the later increase in temperature normal growth was resumed. The check on growth the following day showed that only one root was damaged by this treatment.

In order to determine the effect of the shrinkage alone on "threshold" air at room temperature was dried by passing it over anhydrous calcium chloride and then circulating it through the chamber. An equivalent shrinkage was obtained. Of five roots thus treated, only one gave a lower "threshold" of stimulation while three had a higher "threshold." (There were no exceptions to the results with temperature.) With this evidence plus some checks in which roots were placed in test tubes of water and transferred to the refrigerator for several hours before use, it is considered certain that temperature has the effect described below. It is also true that the difference in the behavior of the same lot of onions in summer and winter is in accord with the other results.

In figure 6, the behavior of an apical five-millimeter segment of root to temperature and current is shown (inset X, figure 4). The current was passed through the root for 30 seconds each time and was alternated up and down. Both 1.50 and 2.00 microamperes stimulated the segment when sent up the root at room temperature (21°–22° C.). When the temperature was lowered to between 10° and 12° C., 1.00 microampere upward current partially stimulated the root and did stimulate it when passed downward. Both

1.50 and 2.00 gave greatly augmented responses when passed up the root but only the latter when passed down. 1.00 microampere again applied at intervals during the next hour stimulated the root when sent in either direction but particularly when sent upward. After an interval, as designated by the time scale, the temperature increased with a resultant fall in the ease of stimulation and during the final period at room temperature it was not

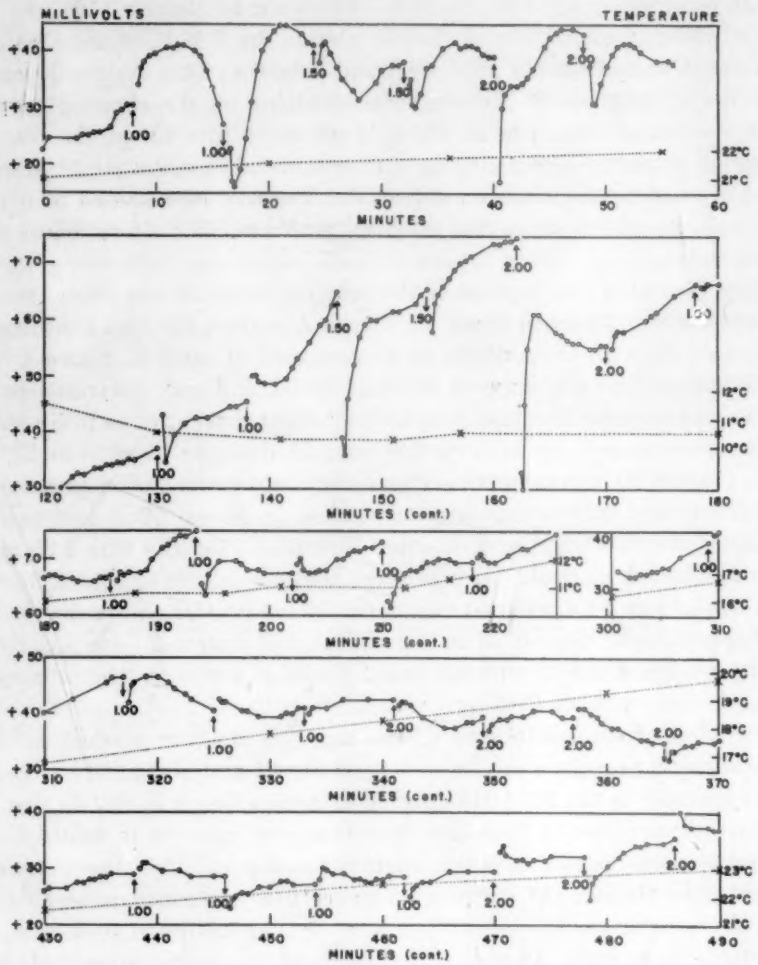


FIG. 6. The effect of temperature on the "threshold" of stimulation. The arrows indicate the direction of applied current up or down the root. The number with each arrow indicates the magnitude of the current in microamperes. The application time was 30 seconds, and the contacts were at 0 and 5 mm. The temperature scale is given on the right, and the variation of temperature with time is given by the broken curve.

possible to stimulate the root as easily as it was at the beginning. Attention is called to the abnormally high P.D. observed in this root at the lower temperatures. This is in contrast to the effect of temperature on potential

reported for the frog skin by LUND and MOORMAN (**10**) and for Valonia by MARSH (**14**). BLINKS (**7**), on the other hand, reports an increase in the potential of Valonia both at low and high temperatures with a minimum P.D. in between the extremes. With an onion root, however, the potential of which is measured in air and is subjected to the drying mentioned above,[4] it is unlikely that any real significance should be attached to the magnitude of P.D. observed at any temperature. As shown by ROSENE (**16**), the presence of water around a part of the root shunts the E.M.F. of these cells and adds or subtracts from the total electrical polarity of the system depending upon the orientation of the electrical gradient of the shunted segment. Since a slight surface film of water is normally present on the root, the change of humidity accompanying temperature alterations might alter the action of temperature alone on potential. The root represented in figure 6 is the only one of this series that exhibited such a marked alteration in inherent potential.

More typical of the response of the polarity potential to a lower temperature are the two curves in figure 7. Curve A is from the first 5 millimeters of the root tip with the contacts as diagrammed in inset X, figure 4. The initial temperature was between 24° and 25° C. and only polarizations and depolarizations were produced respectively when current of as much as 2.00 microamperes passed up or down the root. A decrease to between 12° and 13° C. found 0.50 microampere giving a very large stimulation as an opposing current but only a depolarization when in series. One microampere stimulated the root when sent in either direction. Results with 1.25 microamperes were not as clear. It is believed that the failure to obtain clear-cut stimulations may be attributed to current of too great an intensity polarizing or depolarizing the root after stimulation had occurred (compare fig. 3). A shorter application of current would probably have revealed a response similar to that in curve B below.

The effect of temperature on a basal segment is not as marked as on an apical segment [compare the AC results of BERRY and HOYT (**3**)]. Typical of this behavior is the 10- to 15-millimeter region (inset Z, fig. 4) that was used on several different roots like the one shown in curve B, figure 7. No stimulations were produced by the current passing in either direction at the higher temperatures. At the lower temperature, polarizations became progressively smaller as the current increased by increments of 0.50 from 1.00 to 2.00 microamperes. Partial stimulations of increasing magnitude were doubtless responsible. Note that this is contrary to the condition at both the warmer temperatures. All three downward applications of current at about 11° C. clearly caused a visible stimulation which was rendered obvious by decreasing the time of flow through the root to 5 seconds.

For any given root, it is clearly shown that a reduction in temperature makes the apical region more irritable. The amount of current required for

[4] No consistent potential change in any given direction was observed in the series of experiments with dry air; however, an increased positivity did result in some cases.

the demonstration of this effect varies from root to root even when they have been grown side by side in the aquarium. It is not surprising, therefore, that under these conditions roots are found which are unstimulative with 2.00 microamperes even at the relatively low room temperatures at which

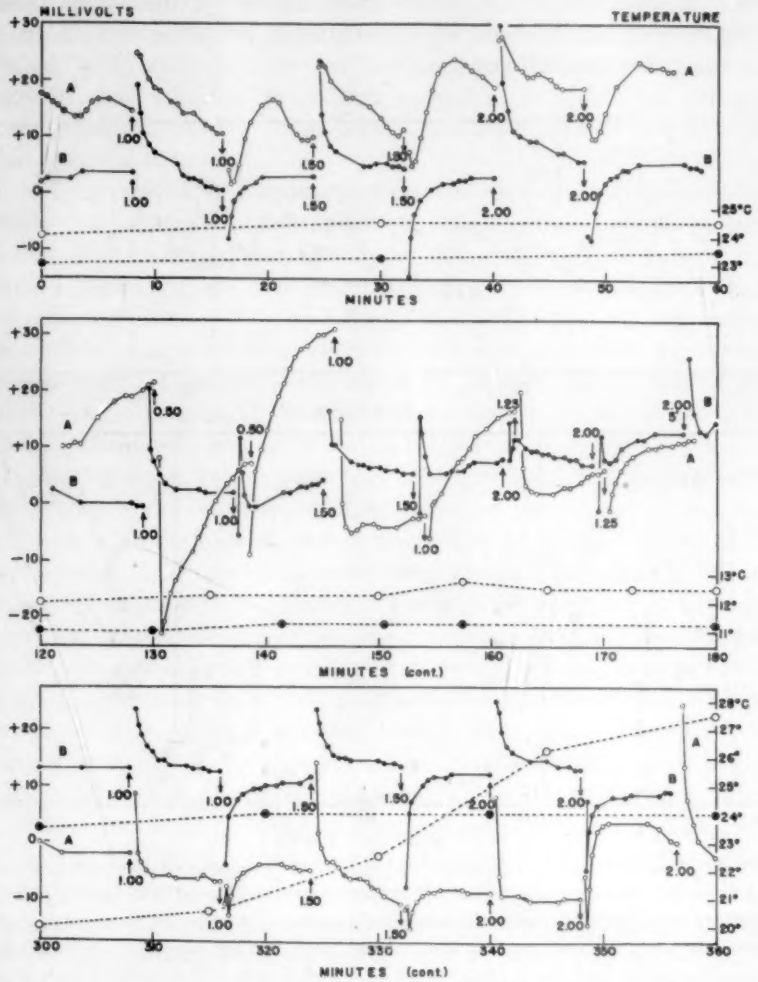


FIG. 7. The effect of temperature on the relative response of apical and basal segments to applied current. The arrows indicate the direction of the applied current. The number with each arrow gives the magnitude of the current in microamperes. Current was applied for 30 seconds and the positions of the contacts were: curve A, 0 and 5 mm.; curve B, 10 and 15 mm. The temperature variation is given by the broken curves. Two separate roots were used for curve A and curve B.

the vast majority of roots are stimulated. In spite of these variations in the behavior of a few roots, it is generally true that larger currents were required for stimulation in summer than in winter. The higher room temperature in Texas coupled with the duration and amount of current employed

probably account for the only occasional observation of stimulation reported in the paper by BERRY (1). Even so, it is difficult to understand why MARSH (11), also working at the University of Texas, failed to find any stimulating effects of currents several times as large as any used in this work. It is not known how much the source and previous history of the onions used for growing roots affects the results but it may well be that some such explanation accounts for the differences.

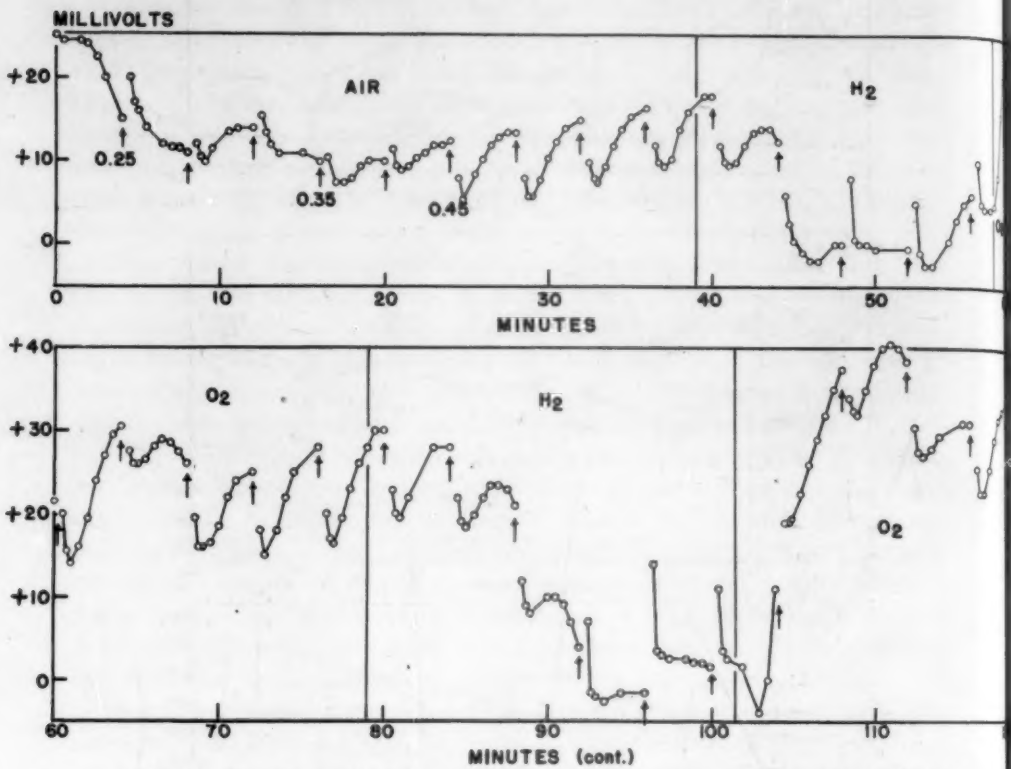


FIG. 8. The effect of hydrogen and oxygen on the response of inherent potential to applied current. The arrows indicate the time at which current was sent up the root. The numbers with the arrows give the magnitude of the current in microamperes. (When no number is given the current was the same as that last indicated.) Current was applied for 30 seconds and the contacts were at 0 and 5 mm. The vertical lines give the time at which the circulation of hydrogen or oxygen was begun.

EFFECT OF HYDROGEN AND OXYGEN ON THE STIMULATING ACTION OF AN APPLIED CURRENT

The amount of current applied up the root was increased until stimulation occurred and then hydrogen gas was passed through the experimental chamber. A drop in observed potential was produced accompanied by a resultant transition from stimulation to polarization after current flow. Typical of this behavior of roots is the curve presented in figure 8. Contacts were at 0 and 5 millimeters above the tip (inset X, fig. 4). Current

was applied for 30 seconds with a 4-minute interval between applications. The perfect reversibility of this action is clearly demonstrated by the repetition of hydrogen administration. The admission of hydrogen and hence the change from stimulation to polarization required a longer period the second time because oxygen had to be replaced rather than air as originally. The action of these gases in producing the changes in inherent potential described by ROSENE and LUND (**18**) is nicely illustrated. The large rebound in oxygen after a period in hydrogen is quite prominent the second time oxygen is admitted. The initial drop that precedes this rise is also commonly observed.

Since the removal of oxygen from the root causes the transition from an irritable to a polarizable condition, it was considered of interest to ascertain the effect of a prolonged period without oxygen on the response of the root to applied direct current. Would it be possible to observe a decrease or loss of the polarizability of the root under these conditions? If not, would the reality of the reported difference in the absolute magnitude of polarization and depolarization as well as the difference between these effects in the absence of any stimulation in apical and basal regions become apparent? The answers to these questions are found in the curves in figure 9. These curves are typical of those found with four different roots. Note that the time scale in this figure has been changed in order to confine the results to a single page with each panel two hours rather than one. The current was passed alternately up and then down the root for 15 seconds and eight minutes elapsed between applications. One fifty-minute gap in readings was allowed for recovery and may be found between 280 and 330 minutes.

With the contacts at 0 and 5 millimeters above the root tip as shown in inset X, the usual change from stimulation to polarization is seen soon after the hydrogen started passing through the chamber, curve A. No appreciable alteration in depolarization accompanied this change. The inherent potential fell to a small negative value during the first 50 minutes of this period and then began to rise slowly throughout the remaining 80 minutes, finally reaching a potential of 10 millivolts positive. From 100 to 160 minutes the polarizations were smaller than the depolarizations but both were increasing in size. While hydrogen was still passing through the chamber, the contacts were raised to 10 and 15 millimeters above the tip of the root (inset Y). It is seen that the polarizations were also smaller than depolarizations (curve B) but the difference was not as pronounced as in the apex. Moreover, the magnitude of the basal polarizations and depolarizations were less than those of the apex. When the contacts were returned to 0.0 and 5 millimeters above the tip with the root still in hydrogen there was no further increase in potential over the next 35 minutes and both polarizations and depolarizations reached a constant and steady value (curve A′); the difference in magnitude of the two persists.

Oxygen was circulated through the chamber beginning just before 240 minutes. Instead of the usual "overshooting" phenomenon which is seen

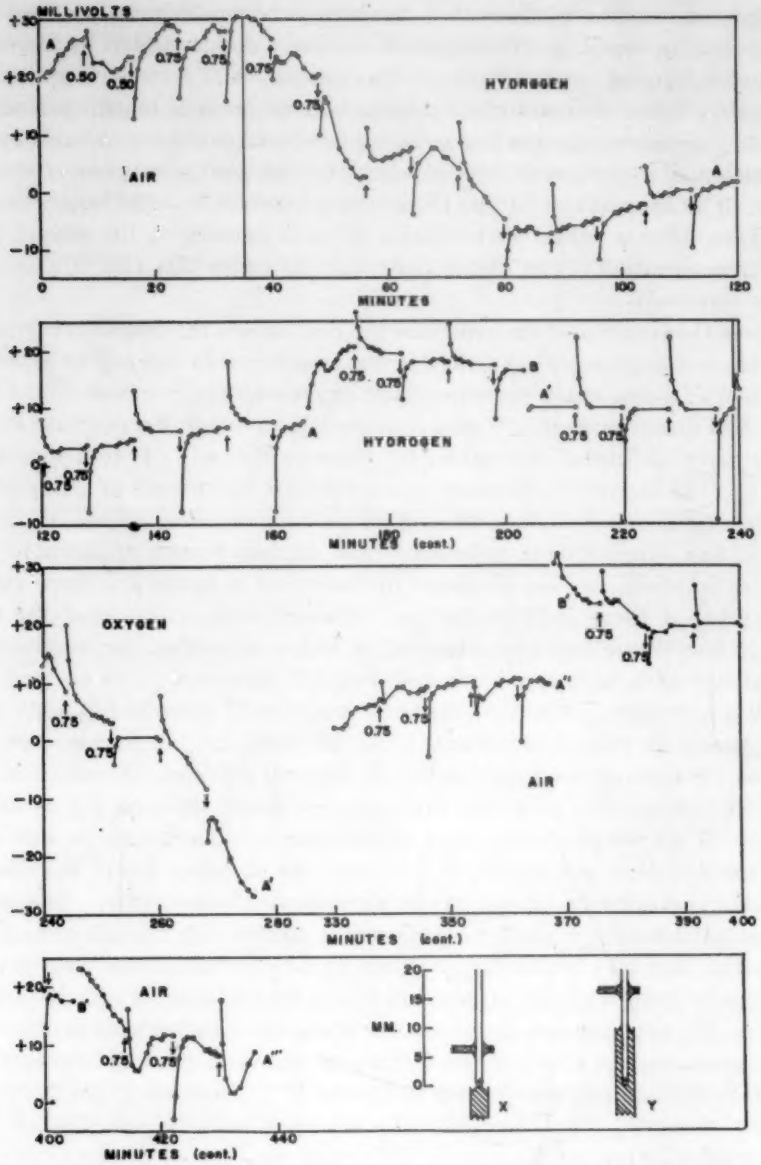


FIG. 9. The effect of hydrogen and oxygen on the relative response of apical and basal segments to applied current. The arrows indicate the direction of the current which was applied for 15 seconds. The numbers give the magnitude of the current in microamperes. (When no number is given the current was the same as that last indicated.) The positions of the contacts were: curves A, 0 and 5 mm. (inset X) and curves B, 10 and 15 mm. (inset Y). The vertical lines give the time at which the circulation of hydrogen or oxygen was begun. All the curves were obtained from the same root.

in figure 8, there was only a small abrupt rise followed by a consistent and appreciable fall in potential that reached nearly 30 millivolts negative over a 35 minute period (curve A′). Moreover, there was no return to stimulation by upward current during this time but the polarizations and depolarizations decreased in size and became more nearly equal. Following the 50-minute recovery period, during which time no readings were made, there was an increase in potential to a positive value as seen in curve A″. The depolarizations were similar to the original ones in air but only a small polarization and a slight stimulation were produced when current passed up the root. The behavior of the basal segment (curve B′) was not very different from that in hydrogen except that the changes in potential produced by the current were essentially equal and slightly smaller than before. The contacts were finally returned to the apex and curve A‴ was obtained. It is seen that there was no complete recovery of the initial irritability but a definite return in that direction was made.

Effect of age on the response of the root to direct current

On three successive days the same root was tested with the same amount of direct current passed alternately up and then down the root for 15 seconds with 8 minutes between each application. The root was returned to the aquarium after each experimental period and permitted to grow. The results from two of the five roots so treated are given in figure 10. The contacts were at 0 and 5 mm. above the tip in all cases. The lengths were as follows: the first root for curve A, 30.4 mm.; curve A′, 37.6 mm.; curve A″, 50.0 mm.; and the second root for curve B, 30.4 mm.; curve B′, 43.8 mm.; and curve B″, 57.3 mm. The diameter of both roots remained unchanged. Remarkably consistent results were obtained in all roots. In every case the only effect of age was a slightly greater change in potential following current flow in the longer, older roots. This took place without any significant difference in the magnitude of the inherent potential. The only root that failed to be definitely stimulated with upward current was the one in curve A when 1.00 microampere was used at 40 minutes. The slight polarization that occurred did not reappear in either A′ or A″. Since the magnitude of the response to 0.50 microampere increased slightly in some cases on successive days it might well be that the "threshold" of stimulation was lowered but this was not tested. It seems reasonable to assume on the basis of these results, however, that the range of root lengths used in this investigation yield quite uniform results.

Discussion

Small direct currents when passed through the root are capable of producing two entirely different types of changes in the observed potentials of the system. One type of change would be expected to occur at any electrode at which electric charges can be separated. Hence the existence of polarization or depolarization following current flow would be anticipated on the

basis of well known physical-chemical facts. The oppositely oriented potential changes following current flow would be expected, however, only as a result of alterations of membranes or phase boundaries which serve as electrodes within the cell. Such an explanation is the well known and generally accepted interpretation of muscle and nerve action potentials in biology.

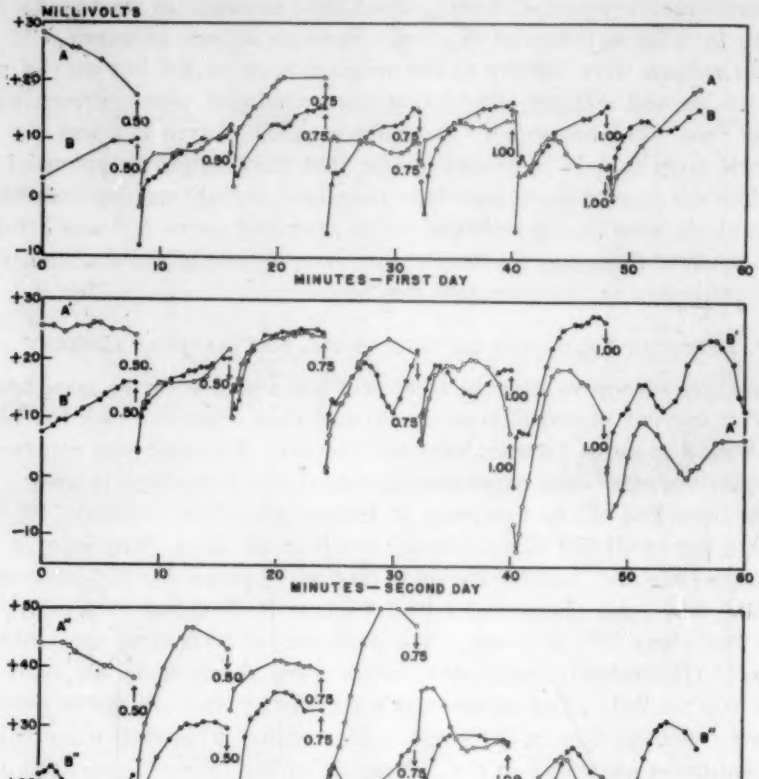


FIG. 10. The effect of root length on the response to applied current. The arrows indicate the direction of the current, which was applied for 15 seconds. The numbers give the magnitude of the current in microamperes. The contacts were at 0 and 5 mm. Curves A, A′, and A″ refer to one root on three successive days, and curves B, B′, and B″ refer to another root on three successive days.

The recovery process in nerve and muscle following a stimulation is thought to consist of restoration of the electrode (plasma membrane) across which the charges are separated. This process has been shown to be dependent on oxidative metabolism. An examination of our results in the light of this

interpretation makes it apparent that additional considerations are required. It may well be that the difficulties are due at least in part to the complexity of a multicellular root as compared to single muscle or nerve cells. It is also true, however, that strict agreement should not necessarily be expected between plant and animal tissue.

For any root capable of being stimulated, it has been shown that the transition from polarization to stimulation is dependent upon the magnitude of the current. If the electrodes involved were the same for polarization and stimulation this would be anticipated. Similarly, if we were dealing with the same electrical system and the response to applied current depended upon the size of the current, this response would be altered by the condition of the system at the time the current was applied. In other words, a stimulated region or one whose potential was reduced to essentially zero by removal of oxygen should not be polarizable. Under both conditions it is shown that polarization is still possible (figs. 4, 5, 8, 9). Also, the polarization that may follow a longer flow of current than that required for stimulation (fig. 3) is in keeping with the above observations. It is not known, however, whether these observations are due to the presence of two different electrical systems within the root, one of them capable of being stimulated and the other only polarizable under the conditions of these experiments, or whether they are due to the same electrical system which is capable of undergoing rapid and marked changes in irritability depending upon the immediate previous treatment of the root. Cells three millimeters or more above the tip are less easily stimulated than those nearer the apex. In fact, with AC stimulation (**3**) it has been found that of the first 5 mm. of the tip only the first millimeter will give a potential change and it is as great a change as that given by the entire 5 mm. for a given amount of current. The polarization immediately following a stimulation or that in hydrogen might very well be explained by a decrease in the potential of cells in the region of the basal contact rather than an increase in the potential of the cells near the apical contact. This interpretation is based on the experiments of ROSENE which show that the E.M.F. observed is an algebraic summation of the E.M.F. of all cells between the contacts. Therefore, with the apical cells stimulated it would be necessary either to reduce the potential of the more basal cells or to increase the potential of the apical cells by means of a non-oxidative, non-irritable system in order for the current flow to increase the potential.

It is certainly clear that stimulation requires oxygen while polarization occurs to even a greater extent in its absence (fig. 9). As in muscles and nerves, the restoration of the "electrodes" which break down during the passage of current depends upon oxidative processes. The work of LUND (**9**), and MARSH (**13**) linking the origin of bioelectric potentials to oxidative metabolism has definitely established that such a dependency exists. The disagreement among the workers in this field concerns the nature of the linkage. This series of experiments requires that any theory must include

some mechanism whereby the intimate dependency between oxygen and stimulation is explained. Similarly, a mechanism that permits polarization and depolarization with or without oxygen and independent of the magnitude or polarity of the inherent potential must be considered. For this last reason, it is thought most likely that within the root it is possible to have both an oxygen sensitive and oxygen insensitive electrical structure or system. The integrity of the electrodes or phase boundaries responsible for polarization and depolarization must be independent of oxygen for several hours at the tension maintained in the experiment. On the other hand, the mechanism of stimulation is inoperative within a short time after the removal of oxygen. According to the discussion above regarding the identity of the two "electrodes" a choice is required between the possibility that all (or most) basal regions have an oxygen insensitive electrical system, which is contrary to the observations of ROSENE and LUND (**18**) even though they found that the apex was more sensitive, or the possibility that the two systems exist simultaneously. The gradual increase in potential which always occurred with a long period in hydrogen after the initial fall particularly points toward the idea that ionic differences independent of any oxidative processes may be at least partially responsible for applied current effects under these conditions.

Summary

1. The response of the inherent potential of onion roots to applied direct current depends upon the direction and magnitude of the current flow. Weak currents passed up the root cause polarizations but as stronger currents are used the potential may decrease. This effect is reversible. Downward currents usually depolarize the root but may, if sufficiently large and under some conditions, increase the potential. There is a marked variation in individual roots as to the size of current required for the different types of response. In fact some roots only polarize and depolarize without exhibiting the stimulative changes. The apical segments of the root, *i.e.*, those involving the first few millimeters of the tip, are more readily stimulated than more basal segments.

2. Short applications of current produce larger stimulations than longer applications. This effect is largely reversible. If the current is applied for sufficient time a polarization may result. Since a stimulated root may be polarized when current is applied before recovery is complete, it is suggested that the total response of the inherent potential to current flow may be an apparent algebraic summation of a stimulation and polarization with the latter increasing as time of flow increases.

3. Temperature of the environment is an important factor in determining the nature of the response of a root to current flow. Lower temperatures render a root more easily stimulated, especially the apical segment. There is considerable variation in the behavior of individual roots in this respect, however.

4. A root which is stimulated in air by current flow can only be polarized in hydrogen. This is perfectly reversible as oxygen replaces the hydrogen and may be repeated several times with consistent results. In the absence of any detectable stimulating effect of current in an atmosphere of hydrogen, it is found that the magnitude of polarizations is less than depolarizations in any given root segment and that both are larger in apical segments than the corresponding change in basal segments.

5. No appreciable change in the response of inherent potential to current flow is produced by growth of the root between 30 and 55 mm. and thus the individual variation of roots may not be attributed to this factor.

6. It is suggested that polarization and depolarization resulting from current flow are due to the accumulation of free ions at "electrodes" within the root. These "electrodes" are not dependent upon oxygen for their integrity nor are they affected by stimulation. On the other hand stimulation must depend upon the breakdown of oxygen-sensitive "electrodes."

It is with pleasure that we acknowledge the technical assistance of Miss Athleen R. Jacobs during part of this investigation.

Bryn Mawr College
Bryn Mawr, Pennsylvania

LITERATURE CITED

1. Berry, L. Joe. Polarization potentials of short duration and their independence of the inherent polarity potential in the onion root. In press.
2. ————————. Spontaneous variations in the electrical potentials of the root of *Allium cepa*. Thesis, University of Texas. 1939.
3. ————————, and Hoyt, Rosalie C. Stimulation of the onion root by alternating current. Unpublished data.
4. Blinks, L. R. The effect of current flow on bioelectric potential. I. Valonia. Jour. Gen. Physiol. **19**: 633–672. 1935.
5. ————————. The effect of current flow on bioelectric potential. II. Halicystis. Jour. Gen. Physiol. **19**: 867–898. 1935.
6. ————————. The effect of current flow on bioelectric potential. III. Nitella. Jour. Gen. Physiol. **20**: 229–265. 1936.
7. ————————. The relations of temperature to the potassium effect and the bioelectric potential of Valonia. Jour. Gen. Physiol. **25**: 905–916. 1942.
8. Clark, W. G. Electrical polarity and auxin transport. Plant Physiol. **13**: 529–552. 1938.
9. Lund, E. J. Relation between continuous bioelectric currents and cell respiration. II. Theory of cell correlation. Jour. Exp. Zool. **51**: 265–290. 1928.
10. ————————, and Moorman, J. B. Electrical polarity and velocity of cell oxidation as functions of temperature. Jour. Exp. Zool. **60**: 249–264. 1931.

11. MARSH, G. The effect of applied electric currents on inherent cellular EMF and its possible significance in cell correlation. Protoplasma **11**: 447–474. 1930.
12. ————. The effect of mechanical stimulation on the inherent EMF of polar tissues. Protoplasma **11**: 497–520. 1930.
13. ————. Kinetics of an intracellular system for respiration and bioelectric potential at flux equilibrium. Plant Physiol. **10**: 681–697. 1935.
14. ————. The effect of temperature on the inherent potential of Valonia. Carnegie Inst. Washington. Pub. no. 475. 1936.
15. REHM, W. S. Electrical response of *Phaseolus multiflorus* to electrical currents. Plant Physiol. **14**: 359–363. 1939.
16. ROSENE, H. F. Proof of the principle of summation of cell E.M.F.'s. Plant Physiol. **10**: 209–224. 1935.
17. ————. The effect of an applied electric current on the external longitudinal polarity potentials of Douglas fir. Amer. Jour. Bot. **24**: 390–399. 1937.
18. ————, and LUND, E. J. Linkage between output of electric energy by polar tissues and cell oxidation. Plant Physiol. **10**: 27–47. 1935.
19. WILKS, S. S. The electric potentials in the coleoptile of *Avena sativa*. Thesis, University of Texas. 1936.

PREPARATION OF SYNTHETIC COMPOSTS FOR MUSHROOM CULTURE[1]

B. B. Stoller

(WITH TWO FIGURES)

Introduction

Horse manure and its straw bedding is the standard medium in commercial mushroom culture today. Since horse manure has become scarce and increasingly costly in our automobile age, attempts have been made to find substitutes during the past twelve years. In order to produce an economical and competitive "artificial manure" or "synthetic compost" it is necessary to use other wastes, especially industrial and agricultural by-products. The method of preparing synthetic composts described in this paper has been tested over a period of five years; several thousand tons have been prepared, and the yield has been as good as or better than that from horse manure composts.

The history of "artificial manures" began in 1923 when Richards and Hutchinson (**5**) patented a method for preparing "nitrogen fertilizers" from straw and inorganic nitrogen. In a later patent (**6**) phosphates also were included. The gist of their method is that 3.6 lb. of nitrogen, preferably in a soluble form, should be added to 500 lb. of straw; larger quantities of nitrogen produced a high alkalinity which checked the decomposition of the straw and caused a loss of nitrogen. In 1930, Lambert (**3**) and Hein (**2**) attempted to use this method for preparing a compost for mushroom culture. The yield of mushrooms they obtained from such composts was less than one-half of the yield from horse manure composts. Their investigation demonstrated that normal mushrooms could be produced from an artificial manure even though the yield was unsatisfactory.

Later Waksman and Reneger (**15**) prepared synthetic composts by adding alfalfa and ammonium phosphate to straw. Their aim in this use of alfalfa was to facilitate the decomposition of the straw by adding to it a "green material" with a high moisture content. The yield of this compost was 70 per cent. of that of a manure compost. They also prepared composts of tobacco stems and straw; the yields of these were variable.

Recently Sinden (**7**) prepared synthetic composts consisting of wheat straw, urea, and wheat. Urea was selected as a source of nitrogen in preference to other compounds because it left "no inorganic residue." The wheat was added to help retain the heating capacity of the compost. The best yields he obtained with such composts were about two-thirds of the yields with manure composts, per square foot of bed surface.

This brief review of the attempts to prepare synthetic composts for mushroom culture shows that only nitrogen has been considered significant, so

[1] This paper is a contribution of the L. F. Lambert Research Laboratory, Coatesville, Pennsylvania.

that the composts prepared by these investigators are essentially the same as the "nitrogen fertilizer" of Richards and Hutchinson. Comparatively slight attention has been given to the need for phosphates and no attempt has been made to observe the necessity for potassium salts. The composts of these investigators refer only to specific mixtures; no means are provided whereby materials other than those specifically mentioned might be substituted. Thus it would be hazardous to deviate from any specific combination since the function of each constituent used is not fully understood. While these investigators have increased our knowledge of synthetic composts, they have not developed a compost which will consistently produce a yield of mushrooms equal to or greater than that produced by a compost of horse manure and its straw bedding.

Basis of present method

The basis for the method of preparing synthetic composts in the present investigation was to analyze both the medium (horse manure) and the product (the mushroom) for their chief chemical constituents, and then seek

TABLE I

AN AVERAGE ANALYSIS OF HORSE MANURE AND ITS STRAW BEDDING

MINERAL CONSTITUENTS	PERCENTAGE, DRY BASIS	AMOUNT IN ONE TON (70% MOISTURE)
	%	*lb.*
N	2.0	12.0
P_2O_5	1.0	6.0
K_2O	2.7	16.0

to produce synthetically a medium which would contain these significant constituents. The composition of horse manure is variable. When numerous samples have been analyzed, however, and when the analyses are compared with those of other investigators, a workable average may be obtained as shown in table I. The analysis of the ash and organic matter of the mushroom is shown in table II. These analyses show that nitrogen, phosphorus, and potassium are present in significant quantities in both the medium and the product, and must all be used in preparing synthetic composts.

As a result of extensive experimentation, it has been found that a ton of synthetic compost with 70 per cent. moisture should contain about 13 lb. N, 4 lb. P_2O_5, and 10 lb. K_2O. It would seem, then, that manure contains more P_2O_5 and K_2O but less N than is necessary to produce the largest yield of mushrooms. The writer believes the reason for artificial manure, in which only the nitrogen factor has been given due consideration, yielding normally "when mixed half and half with composted horse manure" (**4**), is because superfluous quantities of P_2O_5 and K_2O are present in the manure. The ratio of N to P_2O_5 to K_2O found in the mushrooms (6.4 : 2.4 : 4.4) appears to be about the same as has been found satisfactory for preparing synthetic composts. This analysis of the mushroom may, however, reflect

TABLE II

AN ANALYSIS OF THE ASH AND ORGANIC MATTER OF THE MUSHROOM (SPOROPHORE), *Agaricus campestris*

MINERAL CONSTITUENTS OF ASH (1)	PERCENTAGE	ORGANIC CONSTITUENTS OF DRY MATERIAL (13)	PERCENTAGE
	%		%
K_2O	43.94	Ether-soluble portion	5.14
Na_2O	2.31	Hot water-soluble organic matter	42.16
CaO	1.32	Alcohol-soluble portion	7.02
MgO	0.21	Hemicelluloses	13.66
Fe_2O_3	0.24	Cellulose	4.86
Mn_2O_3	0.02	Lignin	0.92
Al_2O_3	2.31	Protein	40.35
P_2O_5	24.25	Nitrogen	6.44
SiO_2	8.23		
Cl	9.22		
SO_3	3.01		

only the specific condition of these constituents in the particular manure on which the mushrooms grew.

The procedure in accordance with the method (10) herein described is to analyze the materials to be used in preparing the synthetic composts into their N, P_2O_5, and K_2O constituents as shown in table III. The straw and root composts are equivalent to a ton of manure with 70 per cent. moisture. In the computation of these composts, the N, P_2O_5, and K_2O in the fibrous material is considered first; any deficiencies of these constituents are then supplied from various sources. The number of sources is immaterial as long

TABLE III

TABULATION OF THE CONSTITUENTS OF SYNTHETIC COMPOSTS

MATERIALS	QUANTITY	N	P_2O_5	K_2O
LICORICE ROOT COMPOST				
	lb.	*lb.*	*lb.*	*lb.*
Spent licorice roots (67.5% moisture)	2000.0	6.5	1.0	.....
Dried brewers' grains	180.0	7.4	1.8	.....
Sulphate of potash (50% K_2O)	18.0	.....	.....	9.0
Muriate of potash (50% K_2O)	3.0	.....	.....	1.5
Superphosphate (20% P_2O_5)	7.5	.....	1.5	.....
Hydrated lime [94% $Ca(OH)_2$]	6.0	.....	.....	.....
Totals	.....	13.9	4.3	10.5
STRAW COMPOST				
Rye straw	500.0	2.5	1.0	5.0
Wet brewers' grains	660.0	7.0	1.6	.....
Uramon (42% N as urea)	8.3	3.5	.....	.....
Superphosphate (20% P_2O_5)	10.0	.....	2.0	.....
Sulphate of potash (50% K_2O)	9.0	.....	.....	4.5
Muriate of potash (50% K_2O)	3.0	.....	.....	1.5
Hydrated lime [94% $Ca(OH)_2$]	9.0	.....	.....	.....
Totals	.....	13.0	4.6	11.0

TABLE IV

THE PREPARATION OF SYNTHETIC COMPOSTS WITH SPENT LICORICE ROOTS AND VARIOUS SOURCES OF NITROGEN. THE RATIO OF $N:P_2O_5:K_2O$ IS ON THE AVERAGE: 15:5:10. SUPERPHOSPHATE (20% P_2O_5) AND SULPHATE OR MURIATE OF POTASH (50% K_2O) ARE USED TO SUPPLEMENT ANY DEFICIENCIES OF PHOSPHORUS AND POTASSIUM IN THE ROOTS AND SOURCES OF NITROGEN TO ATTAIN THIS RATIO

PLOT	SOURCES OF NITROGEN	ADDED PER TON OF ROOTS	NITROGEN PER TON OF ROOTS	SIZE OF PLOT	MUSHROOMS PER PLOT	MUSHROOMS PER SQ. FT.	COMMERCIAL YIELD* PER SQ. FT.
		lb.	*lb.*	*sq. ft.*	*lb.*	*lb.*	*lb.*
38	Dried blood	60.0	7.8	48	111	2.31	1.73
40	Cottonseed meal	120.0	8.0	48	113	2.35	1.77
41	Soybean meal	123.0	8.0	48	141	2.94	2.20
42	Castor bean meal	145.5	8.0	48	129	2.69	2.02
43	Malt sprouts	189.5	8.0	48	164	3.42	2.56
46	Beet molasses	200.0	2.8	48	92	1.92	1.44
	Ammophos	20.0	3.2				
	Urea	4.4	2.0				
47	Tobacco stems (ground)	150.0	3.8	48	97	2.02	1.52
	Urea	10.0	4.5				
48	Cottonseed meal	40.0	2.7	48	129	2.69	2.02
	Dried blood	20.0	2.6				
	Urea	5.5	2.7				
49	Dried blood	7.7	1.0	24	64	2.67	2.00
	Cottonseed meal	15.0	1.0				
	Soybean meal	15.4	1.0				
	Dried brewers' grains	23.4	1.0				
	Dried yeast	2.5	0.2				
	Urea	8.6	4.0				
49A	Same as 49	.....	.....	24	61	2.54	1.91
51	Dried tannery sludge	200.0	4.0	48	119	2.48	1.86
	Urea	8.6	4.0				
52	Same as 51 plus "Ultra-Life" —(Vitamin feed conc.)	10.0	.....	48	131	2.73	2.05
53	"Uramon" (urea with 42% N)	20.4	8.5	24	27	1.13	0.84
53A	Same as 53 plus 2 lb. more lime	.....	.....	24	47	1.96	1.47
54	"Uramon"	22.8	9.5	48	143	2.98	2.24
	Chopped straw	75.0					
	Cheese whey	10.0					
55	Calcium nitrate	63.5	9.5	48	136	2.83	2.13
58	Ammonium sulphate	42.5	8.5	48	None	.....	.....
59	Ammonium sulphate	47.5	9.5	24	59	2.46	1.84
	Chopped corn cobs	100.0					
	Cheese whey	10.0					
59A	Same as 59 plus 2 lb. more lime	.....	.....	24	39	1.63	1.22
60	Dried brewers' grains	200.0	8.2	48	151	3.15	2.36
66	Same as 60 plus different source of potash	200.0	8.2	48	148	3.08	2.31
70	Dried brewers' grains	100.0	4.1	24	71	2.96	2.22
	Urea	9.2	4.2				
75	Dried brewers' grains	100.0	4.1	48	157	3.27	2.45
	Dried blood	7.7	1.0				
	Cottonseed meal	15.0	1.0				
	Soybean meal	15.4	1.0				
	"Uramon"	4.8	2.0				

TABLE IV—(*Continued*)

PLOT	SOURCES OF NITROGEN	ADDED PER TON OF ROOTS	NITROGEN PER TON OF ROOTS	SIZE OF PLOT	MUSHROOMS PER PLOT	MUSHROOMS PER SQ. FT.	COMMERCIAL YIELD* PER SQ. FT.
76	Same as 75 plus 6 lb. more lime	.....	.....	48	163	3.40	2.55
77	Same as 75 plus 6 lb. more of magnesium lime	.....	.....	48	139	2.90	2.17
78	Extracted cocoa cake	200.0	6.0	48	147	3.06	2.30
	Urea	7.6	3.5				
79	Extracted cocoa cake	100.0	3.0	48	129	2.69	2.02
	Urea	13.0	6.0				

* Commercial yield: Since the mushrooms, as weighed for the cannery, included the stubs, a reduction of 25% is allowed, so as to conform with the weight of mushrooms cut for the market. The average weight of the stubs, with adhering soil, is 22% of the weight of the mushroom.

as they add up to the total quantities and ratio desired. Synthetic composts prepared from many different sources of N, P_2O_5, and K_2O gave equally good yields. This fact shows that these three constituents are available for the most part to the mushroom mycelium in the various sources investigated. The writer has observed large particles (of chemicals), which were very insoluble in water or in agar medium, oxidized by the growing mycelium.

In preparing the compost pile, the nitrogenous material is spread or sprayed over the surface of the widened heap of fibrous material and forked in about a foot. All mineral ingredients are then mixed together, diluted with an equal quantity of loam, and spread evenly over the top of the heap. The whole mass is then mixed, watered as it is turned, and arranged into a pile which will provide the necessary aeration.

This method of preparing composts by analyzing and tabulating the various materials affords a formulation in which various agricultural and industrial by-products may be substituted. In order to compete with a waste product like manure, the possibility for substitution is important; it is necessary to have a formula in which the least expensive by-products on the market may be used. This method also establishes a basis for the estimation of the quantity of a material required in a synthetic compost.

Materials for synthetic composts

Since the mushroom is a fungus, an organic source of carbon has to be supplied. Straw which has undergone a microbial decomposition is a satisfactory source. The straw also provides for the mushroom bed the fibrous structure which is necessary for the intensely aerobic growth of the mushroom mycelium. Fibrous materials like spent licorice roots (from which the licorice has been extracted) and spent tannery nuts, bark, and leaves (from which tannin has been extracted for industrial use) are even more satisfactory than straw. These materials already contain sufficient moisture, are suitably subdivided, and require only a short or no microbial decomposition.

The use of numerous nitrogen sources for synthetic composts is shown in table IV. Composts with some nitrogen sources gave better yields than with others. The yields, however, were affected by the composting period which, at 30 days, was rather long for these small plots; in a later consideration of the problem of composting it seemed that the yields of some of the plots would have been better if the composting period had been shorter.

Organic nitrogenous materials are preferable to inorganic because they serve also as a source of carbon, contain some K_2O and P_2O_5, and have a better heating capacity. A solution of inorganic nitrogenous substances absorbed in very finely ground straw or corn cobs, however, is a fair equivalent to the organic material. The selection of a suitable nitrogen source also depends, of course, on the cost of the material compared to the yield of mushrooms obtained by its use. The quality of the mushrooms produced by the use of any specific material is not superficially evident; possible intrinsic values are not commercially important at present.

Some organic nitrogenous materials may be unsatisfactory due to the presence of specific toxic constituents as in the case of cocoa shells and cake. These materials contain one per cent. or more theobromine which, at this concentration, prevents the growth of the mushroom mycelium. When the theobromine is extracted, however, as is the practice in the commercial manufacture of theobromine, the resulting extracted cocoa cake is a satisfactory source of nitrogen.

In the use of inorganic nitrogenous substances like ammonium salts, cyanamide, and urea special precautions are necessary; but nitrates may be used freely. Ammonia formed from the former substances persists in the compost (due to adsorption) even after the "sweating out process" and prevents the growth of the mushroom mycelium. One of the simplest methods for suppressing this excessive ammonification is to absorb solutions of the ammoniating substances in finely ground carbonaceous materials as already indicated; in this way the transformation of ammonia to microbial proteins is assisted; the use of an acidifying material like cheese whey is also helpful. If calcium cyanamide is improperly stored, that is, exposed to air so that it absorbs moisture and carbon dioxide, it is transformed to calcium dicyanodiamide. Composts prepared with dicyanodiamide even prevent the growth of the mushroom mycelium. In contrast to ammoniating salts, nitrates are not toxic to the mycelium. Nitrates are especially suitable for use with fibrous materials which do not undergo extensive microbial decomposition. Composts of nitrates with spent licorice roots or spent tannery bark require no outdoor composting.

The variety of sources of the other materials employed in the preparation of synthetic composts is comparatively limited. The best source of phosphates is superphosphate. Besides the fertilizer grades of potassium chloride and sulphate, the ash of various plant materials such as cottonseed hulls, and the ash of the sludge of alcohol distilleries may also be used. Current investigations, which will be reported at a future date, indicate that the

"minor elements" are related to disease control. The addition to composts of yeast and vitamin concentrates failed to significantly increase the yield; the vitamin content of the mushroom, however, may have been increased.

TABLE V

THE EFFECT OF POTASH, PHOSPHATE, AND LIME ON THE YIELD OF MUSHROOMS WHEN ADDED TO A SYNTHETIC COMPOST CONSISTING OF SPENT LICORICE ROOTS AND BREWERS' GRAINS

Plot	Fertilizer salts per ton of compost				Mushrooms per 24 sq. ft.	Mushrooms per sq. ft.	Average
	K_2SO_4, 50% K_2O	KCl, 50% K_2O	Superphosphate, 20% P_2O_5	Hydrated lime, 94% $Ca(OH)_2$			
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
61	18	3	5	None	65	2.71	2.6
81					58	2.42	
62	18	3	None	6	52	2.16	2.1
82					50	2.10	
63	None	None	5	6	38	1.60	1.4
83					27	1.13	
64	18	3	5	6	55	2.30	2.4
84					59	2.46	
65	18	3	5	3	60	2.50	2.6
85					62	2.60	
66	18	3	5	9	58	2.42	2.4
86					57	2.38	
67	18	3	2.5	6	74	3.10	2.7
87					56	2.34	
68	18	3	7.5	6	50	2.10	2.3
88					59	2.46	
69	12	2	5	6	48	2.00	2.1
89					54	2.25	
70	24	4	5	6	49	2.04	2.0
90					49	2.04	
71	None	None	None	None	32	1.33	1.1
91					21	0.88	
72	"	"	"	6	21	0.88	0.8
92					16	0.67	
73	"	"	5	None	31	1.30	1.4
93					35	1.46	
74	18	3	None	"	50	2.10	2.1
94					52	2.16	
75	None	None	"	"	22	0.92	0.9
95					23	0.96	
Manure C	"	"	"	"	52	2.16	
" D	"	"	"	"	50	2.10	2.3
" E	"	"	"	"	60	2.50	
" F	"	"	"	"	56	2.34	

The effect of potash

Synthetic composts of spent licorice roots as shown in table III afford an excellent opportunity to test the effect of potash on the yield of mushrooms. Practically all of the potash has been washed out of the licorice roots during the hot water extraction; similarly, most of the potash is removed from the brewers' grains during the extraction of the malt. Furthermore, the spent

licorice roots are ideal for plot experiments; the roots have been cut into 1- to 2-inch lengths and vary from fine threads to fibers $\frac{3}{8}$ inch in diameter. The roots retain moisture evenly and handle easily. An eight-ton mixture of the roots and brewers' grains was prepared and composted for a month. At the end of this time the pile was divided into 16 heaps; potash, superphosphate, and lime were added to these heaps; they were then thoroughly mixed and split into two plots as shown in table V. The duplicate plots were placed on different shelves in the mushroom house.

The results of the effect of potash are shown in table V. Due to overcomposting and cooling of the compost when making up the plots, the mushroom house did not heat properly during the sweating out process. As a consequence, insect trouble was encountered and the yields were not altogether satisfactory. The effect of the addition of potash, however, is clear: by its use the yield was almost double that of plots to which only N and P_2O_5 were added. Other experiments with plots containing a ton of compost substantiate this conclusion. The effect of adding phosphates is not distinct because some three-fourths of the phosphates required were already present. Lime had no effect on the yield under the conditions of this experiment.

Composting

After all the necessary ingredients are supplied the next important step is the fermentation or "composting" of the materials. The process of composting consists in the microbial decomposition of the carbonaceous materials, in the synthesis of microbial proteins, and in the conditioning of the fibrous materials to absorb and retain moisture. WAKSMAN and NISSEN (**14**) have shown that as a result of the composting of manure there is a reduction in water-soluble substances, hemicelluloses, and to less extent in cellulose. This is accompanied by an increase in lignin, total nitrogen (which is present chiefly in the form of insoluble microbial proteins), and ash. The experiments of WAKSMAN and IYER (**12**) indicate that lignin and microbial proteins form complexes. It appears that the nitrogen of these ligno-protein complexes is unavailable unless the lignin is oxidized. It is assumed that the mushroom mycelium can oxidize lignin because the lignin content of the compost decreases as a result of the growth of the mycelium. The writer (**11**) has observed that the mycelium forms highly colored products when lignin, tannin, and many aromatic hydroxy and amino compounds are added to an agar medium. These colored products appear to be the result of oxidations by the mycelium. The mushroom mycelium has strong oxidizing powers which enable it to utilize the insoluble nitrogen of the ligno-protein complexes by oxidizing the relatively toxic lignin. Since the mycelium has a nitrogen source at its disposal which is unavailable to most of the microorganisms present in the compost heap, it is able to consume the carbonaceous materials not used previously.

The length of the composting period is of paramount importance, although the determination of the length is still an art. The experienced

grower considers the composting sufficient when the manure has a dark color (indicating the liberation of lignins by the consumption of other constituents) and when the tensile strength of the straw in the manure is low (indicating that the straw can absorb and retain water). From the point of view of preparing synthetic composts the length of the composting period is influenced by the nature and subdivision of the fibrous material, by aeration of the compost pile, and by the use of lignin and tannin extracts. These factors will now be considered.

When the fibrous material is the same as that present in manure composts, *i.e.*, straw, then the composting period is relatively long—about 4 to 5

TABLE VI

THE EFFECT OF CHOPPING THE STRAW OF SYNTHETIC STRAW COMPOSTS ON THE YIELD OF MUSHROOMS (AS SHOWN IN TABLE III)

PLOT	LONG STRAW	CHOPPED* STRAW	BED SURFACE	MUSHROOMS PER PLOT	MUSHROOMS PER SQ. FT.	COMMERCIAL YIELD PER SQ. FT.
	lb.	*lb.*	*sq. ft.*	*lb.*	*lb.*	*lb.*
1	2000	2000	696	2484	3.57	2.7
2	2000	None	384	1273	3.32	2.5
4	250	250	48	142	2.96	2.2
4A			48	142	2.96	
5	None	500	48	98	2.04	1.7
5A			48	115	2.40	
12–2	200	250	36	96	2.67	2.1†
12–2A			45	132	2.93	
12–3	None	450	39	111	2.85	2.1†
12–3A			36	103	2.84	
12–4	450	None	50	101	2.00	1.8†
12–4A			50	138	2.76	
Check	Manure	.....	3600	12003	3.33	2.5

* Straw was chopped with a hammer mill, using a one inch screen.
† These results are typical of 4 or more replications in which different nitrogen sources were used.

weeks. It is necessary to cut the straw in order to facilitate the retention of heat and moisture both during the composting period and during the "sweating out process" (**8**). Cut straw is also easier to handle; long straw binds and is laborious to turn. By subdivision of the straw the length of the composting period may be reduced. Before adding the various ingredients to the straw, its moisture content is conveniently brought up to about 50 per cent. by placing a revolving lawn sprinkler on top of the straw pile, which should be about 6 ft. high. It is sprinkled until water leaches from the pile; the watering is continued for several days. Rye and wheat straw are preferable to oat straw or corn stover since the latter have a tendency to lose their fibrous structure when the microbial decomposition has proceeded to the stage where moisture is retained well.

The effect of chopping the straw on the yield of mushrooms is shown in table VI. The results show that chopping the straw caused a reduction in the number of square feet of bed surface and also a reduction in the yield for a given quantity of straw. The reason for the plots with the entire straw chopped giving less mushrooms per plot is because all the plots were composted for an equal length of time; the plots with all chopped straw composted faster than the plots in which the straw was partially or not chopped. When the chopped straw was arranged in plots with the *same* bed surface area as the partially chopped straw (plots 4 and 5), the yield per sq. ft. of bed surface was greatly reduced. If allowance was made for the shrinkage due to the rapidity of composting (all the beds made up to a six-inch depth regardless of the resulting surface area) and if the composting period was shorter as in plots 12–2,3,4, then there is no marked difference in yield.

TABLE VII

THE EFFECT OF THE LENGTH OF COMPOSTING AND THE VENTILATION OF LICORICE ROOT COMPOST ON THE YIELD OF MUSHROOMS

PLOT	DATE OF ARRANGEMENT OF PLOT, 1938	NUMBER OF DAYS COMPOSTED	NUMBER OF TIMES COMPOST WAS TURNED	COMPOST IN PLOT WHEN ARRANGED	BED SURFACE	MUSHROOMS PER PLOT	MUSHROOMS PER SQ. FT.	COMMERCIAL YIELD PER SQ. FT.
				lb.	*sq. ft.*	*lb.*	*lb.*	*lb.*
B–1	Aug. 18	16	3	950	48	167	3.48	2.60
B–2					48	163	3.40	2.54
B–5	Aug. 25	8	2	950	48	187	3.90	2.92
B–6					48	179	3.73	2.79
B–7	Sept. 1	2	1	950	48	115	2.40	1.79
B–8					48	80	1.67	1.25
B	Aug. 17	17	3	25000*	1416	2871	2.03	1.52
2†	Oct. 26	21	3	22000*	1152	4336	3.76	2.82

* Approximately. Each of these piles is typical of 3 others prepared at the same time.

† Ventilator placed in pile at the second turning.

Even though a larger yield for a given quantity of material is obtained from composts of long straw, the best commercial practice is to cut the straw in view of the disadvantages of using long straw.

The composting period is comparatively brief when the fibrous materials are spent licorice roots or spent tannery bark, nuts, and leaves. The reason for these materials requiring less composting is that their chemical composition is more or less similar to composted manure; *i.e.*, a large percentage of lignin or tannin and cellulose, and a small amount of easily decomposable carbonaceous matter. These materials contain about 65 per cent. moisture when purchased, and have other desirable qualities already described. They make better composts than straw.

Synthetic composts prepared with spent licorice roots and composted for 15 days in 1937 gave commercial yields of 2.5 lb. per sq. ft. of bed surface, when prepared in plots of a ton or less. As a result, large piles of licorice

root compost, as plot B in table VII, were prepared the next year. The yields of the large piles were unsatisfactory; however, small plots (B1 to B8, table VII) arranged at the same time to determine the length of the composting period gave good yields. It was evident, then, that the composition of the synthetic compost was satisfactory, but that the method of composting was improper. The small piles had a relatively large surface exposure and only a small "core" removed from the atmosphere, so that almost all parts of the small piles received good aeration; the reverse was the condition present in the large piles. By placing a ventilator, made of a lattice-work of

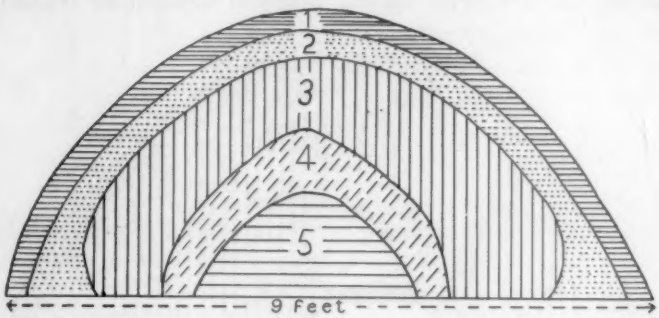


FIG. 1. Cross section of synthetic licorice root compost pile without ventilator. Measurements were made after composting 12 days, at the second turning.

ZONES	MOISTURE	pH	TEMPERATURE	SIDE OF PILE		TOP OF PILE
				CO_2	O_2	CO_2
	%		°F.	%	%	%
1. Wet-ammonia	75	8.2	120	2.5	17.4	8.0
2. Fire fang	63	7.9	140	7.8	12.1	11.0
3. Dark brown	64	8.1	165	14.1	5.8	15.5
4. Sour-green	64	5.8	160	24.3	0.2	22.0
5. Very sour-green	66	5.2	140	24.9	1.6	22.2

wood, in the center of the pile good aeration was also obtained in the large piles. The yields from the aerated piles was satisfactory as shown in plot 2, table VII. The conditions of moisture, pH, temperature, and aeration before and after placing a ventilator in the large piles is shown in figures 1 and 2. When preparing composts with straw the problem is to compress the straw as much as possible in order to prevent too much aeration from drying out and cooling the straw compost, whereas with closely packed materials like licorice roots it is necessary to make special provisions for aeration. STOLLER, SMITH, and BROWN (9) demonstrated that manure could be composted in about 7 days when good aeration was provided.

The results in table VII show that the best yields are obtained with small piles of licorice root composts by composting for 8 days. Composting twice as long reduces the yields somewhat; composting only 2 days reduces the

yields drastically and encourages all kinds of mold growth. The large quantity of nitrogen used in preparing these synthetic composts necessitates a composting period of some 8 days. While 8 days of composting suffices in small piles, about 15 to 20 days are required in large piles with ventilators. It is not as simple to obtain proper mixing, watering, and aeration in large piles as it is in small ones.

Since one of the chief functions of composting is to make the nitrogen unavailable to most microorganisms by its combination with lignin, the possibility occurred of achieving this combination without the lengthy microbial decomposition. Accordingly, several nitrogen sources as shown in table

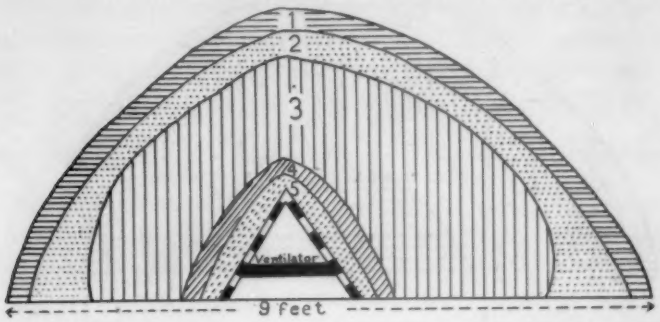


FIG. 2. Cross section of synthetic licorice root compost pile with ventilator. Measurements were made after composting 12 days, at the second turning.

ZONES	MOISTURE	pH	TEMPERATURE	SIDE OF PILE		TOP OF PILE
				CO_2	O_2	CO_2
	%		°F.	%	%	%
1. Wet-ammonia	75	8.2	120	3.5	17.5	4.5
2. Fire fang	58	7.7	140	12.0	8.3	4.0
3. Dark brown	66	8.0	160	9.0	11.1	3.0
4. Dry	66	7.7	.....	4.5	15.7	1.8
5. Fire fang	63	7.9	140	2.4	18.4	0.7

VIII were soaked in solutions of lignin extracts of the paper industry and in solutions of tannin extracts. Exact details of the procedure are described elsewhere (**10**). Substances like lignin or tannin are called "coprinating" agents.

The results in table VIII show that when concentrated nitrogenous materials are soaked in solutions of lignin or tannin extracts, the outdoor composting process is unnecessary. In most cases the yields were better without than with composting when lignin or tannin was used. Some nitrogenous materials gave better yields with tannin than with lignin, and vice versa. It is interesting to note that the plots with "Uramon" (a Dupont product in which the urea is diluted to 42 per cent. N) gave a good yield of mushrooms without composting when combined with lignin and tannin.

TABLE VIII

SYNTHETIC LICORICE ROOT COMPOSTS PREPARED BY THE COMBINATION OF TANNIN OR LIGNIN WITH NITROGENOUS MATERIALS. EACH PLOT HAS A BED SURFACE OF 48 SQUARE FEET

Nitrogen sources	Nitrogen per ton of licorice roots	"Coprinating" agent	Agent per ton of licorice roots	Plots composted 30 days out-of-doors			Plots composted 7 days out-of-doors			Plots not composted out-of-doors			
				Plot	Mushrooms per plot	Mushrooms per sq. ft.	Plot	Mushrooms per plot	Mushrooms per sq. ft.	Plot	Mushrooms per plot	Mushrooms per sq. ft.	Commercial yield per sq. ft.
	lb.		*lb.*		*lb.*	*lb.*		*lb.*	*lb.*		*lb.*	*lb.*	*lb.*
Soybean meal	115	Tannin	30	28	127	2.65	31	151	3.15	41	171	3.56	2.67
" "	115	Lignin	45	29	113	2.35	32	139	2.90	42	103	2.15	1.60
Dried brewers' grains	180	"	50	30	127	2.65	33	148	3.08	43	153	3.19	2.40
Brewery sludge	350	None	.....	26	110	2.29	34	148	3.08	44	164	3.42	2.52
Wet brewery yeast	450	"	.....	.....	.....	.....	35	94	1.96	.....	.....	.....	.....
" " "	450	Tannin	30	.....	.....	.....	36	146	3.04	46	159	3.31	2.50
Dried blood	60	"	25	15*	107	2.23	.....	.....	.....	47	131	2.73	2.04
" "	60	Lignin	40	.....	.....	.....	38	149	3.10	48	125	2.60	1.96
Uramon (42% N as urea)	18	"	47	3†	88	1.84	39	141	2.94	49	140	2.92	2.19
" " " " "	18	Tannin	30	4†	94	1.96	40	140	2.92	50	146	3.04	2.29

* No tannin in this plot.
† Composted 42 days out-of-doors.

Since urea is very toxic to the mushroom mycelium, it may be inferred that a real combination did occur between it and the lignin or tannin. The plot with yeast combined with tannin showed a much better yield than with yeast alone. The probable reason why plots with the brewery sludge alone gave a good yield is because it consists of the proteins of the malt which are precipitated by the tannins of the hops; actually, then, these plots of brewery sludge did contain tannin. Sometimes, under the conditions of indoor composting, fair yields were obtained when no lignin or tannin extracts were used. But usually such plots ammoniated strongly so that the mycelium could not grow, or molds developed and prevented the growth of the mycelium. Indoor composting of synthetic composts requires the use of substances in the *nature* of lignin or tannin and fibrous materials like spent licorice roots or spent tannery bark. The obstacle to the use of this process is that the solutions of tannin and lignin are necessarily acid; although the finished compost has a pH of 7.0 or higher the presence of acidifying substances makes such composts more susceptible to the truffle disease of the mushroom mycelium to be described later.

Preparation of the mushroom bed

In order to obtain good yields it is not only necessary to consider the composition and condition of the compost, but also the quantity used in preparing the mushroom bed. The usual commercial practice is to use about 12 bushels of compost for 24 sq. ft. of bed surface, so that the beds have a depth of about 6 inches after tamping. A licorice root compost, which is an ideal material for experimentation, as already explained, was used to prepare beds in which the number of bushels for 24 sq. ft. of bed surface was varied from 6 to 14 as shown in table IX. The results show that the yield increased in almost the same proportion as the increase in quantity of compost; the yield from 12 bushels was almost double that from 6 bushels. In another experiment the volume or depth of the bed was kept constant by diluting one-half of the root compost with an inert material like spent Quebracho wood chips; the result was that the yield was reduced to about one-half. Thus it appears that the usual commercial practice of making the beds gives the best yields.

In table IX there is shown also how the bed temperatures vary with the quantity of material present in the bed. The bed temperature may be controlled by regulating the quantity of material in the bed. It is necessary to attain temperatures of 140° F. in the beds during the "sweating out process" in order to obtain air temperatures of 125° to 130° F. in the mushroom house for the purpose of killing pests. The plots with 6 to 8 bushels per 24 sq. ft. (table IX) were free of pests because the air temperature was 125° to 130° F. since most of the beds in the mushroom house had 12 bushels, and consequently, a temperature of 140° F. It is evident, then, that about 12 bu. of compost should be used for 24 sq. ft. of bed surface, not only to obtain the best yields but also to obtain the high temperatures necessary for killing pests.

TABLE IX

THE EFFECT OF QUANTITY OF SYNTHETIC LICORICE ROOT COMPOST PER SQUARE FOOT OF BED SURFACE ON THE TEMPERATURE DURING THE "SWEATING OUT PROCESS" AND ON THE YIELD OF MUSHROOMS

Plot	Temperatures of "sweating out process"		Size of plot	Bushels† for 24 sq. ft.	Compost used when filling beds, 66% moisture		Mushrooms from 24 sq. ft.	Mushrooms per sq. ft.‡	Dry wt. basis		Commercial yield per sq. ft.
	Highest temp. attained	Av. temp. during 72 hrs. of peak heat*			In 24 sq. ft.	Per sq. ft.			Compost per sq. ft.	Mushrooms per sq. ft.	
	° *F.*	° *F.*	*sq. ft.*	*bu.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
1	130	126	24	6	252	10.5	41	1.67	3.57	0.167	1.25
5	133	129					39				
2	135	130	24	8	336	14.0	55	2.23	4.76	0.223	1.65
6	133	130					52				
3	141	137	24	10	420	17.5	58	2.42	5.95	0.242	1.80
7	139	136					58				
4	145	140	24	12	504	21.0	77	3.20	7.14	0.320	2.40
8	145	141					76				
9	148	146	24	14	588	24.5	82	3.42	8.33	0.342	2.55
Bed 8	.....	.....	312	8	(336)	(14.0)	53	2.20	(4.76)	0.220	1.65
Pile 5	146	139	1392	12	(504)	(21.0)	78	3.23	(7.14)	0.323	2.40

* Average of six successive readings at 12-hour intervals.
† The average bushel weighed 42 lb.
‡ Average.

pH of the compost and the control of weed and disease fungi

The optimum pH for the growth of the mushroom mycelium is 6.5 to 7.0; this pH range, however, is very suitable for other fungi so that the pH of the compost must be adjusted to prevent weed and disease fungi. The common green molds which are omnipresent on spoiled food, and which have spores that are resistant to high temperatures, are easily prevented from growing on the compost by maintaining the pH above 7 0. The many fungi that can grow on composted manure or synthetic composts when the pH is above 7.0 are all eliminated by the high temperatures attained during the "sweating out process" with the exception of two: Coprinus sp. (chiefly *Coprinus fimetarius*) and *Pseudobalsamia microspora.*

The conditions are ideal for Coprinus sp. when there are sufficient alkaline minerals in the compost to cause a small liberation of ammonia from an abundant supply of nitrogenous matter in the compost and, as a consequence, the pH is 8.0 to 9.0. Ammoniating salts and urea are not as toxic to it as to the mushroom mycelium. The addition of one liter of 50 per cent. ammonium hydroxide to 5 sq. ft. of bed surface during the "sweating out process" encouraged the growth of Coprinus sp., whereas even 10 per cent. NH_4OH prevented the growth of the mushroom mycelium, though there was little change in pH. The control of Coprinus sp. would be achieved by acidifying the compost to pH 7.0–7.5, if this procedure were not favorable to the other heat resistant fungus—*P. microspora.*

Since very little is known about *P. microspora,* which causes the truffle disease of the mushroom mycelium, it is necessary to discuss it in some detail. *P. microspora* cannot grow on a *non-sterile* manure or synthetic compost, even if the compost is acidified so that the pH is reduced to 6.0. It can grow only in the presence of or, more likely, on the mushroom mycelium. The writer (**11**) has shown that it is one of the few fungi which can produce volatile sulphides. It seems that with the implement of these sulphides this fungus can attack the mushroom mycelium. A quinone-like substance continually volatilizes from the surface of the mushroom mycelium as a result of its intense oxidizing activity; this volatile substance acts as a barrier to all organisms, except insects and *P. microspora,* that attack the mushroon mycelium (**11**). The sulphides of *P. microspora* enable it to reduce the quinones so that it can attack, or at least grow in the presence of, the mushroom mycelium. The reducing ability of *P. microspora* may be demonstrated when a chemical like alpha naphthol is added to an agar medium and inoculated first with the mushroom mycelium, which causes the agar to be colored purple, and then inoculated with *P. microspora,* which then decolorizes the agar.

The growth of *P. microspora* on the mushroom mycelium is expedited by the presence of acidifying substances and sulphur compounds in the compost, even though the overall pH may be 8.0. The mushroom mycelium produces acids which reduce the initial pH 8.0–8.5 of the compost to 6.0–7.0. It is possible that the presence of acidifying materials in the compost assists the evolution of sulphides by *P. microspora* after the pH of the compost has been

reduced by the mushroom mycelium. It has been observed that the addition of acidifying substances like mineral acids, gypsum, superhosphate, tannic acid, etc., to the compost permits the rapid growth of *P. microspora* on the ensuing mushroom mycelium. When acidifying substances are absent and when the compost is properly buffered with alkaline salts, the mycelium of *P. microspora* is greatly retarded in its growth on the mushroom mycelium and the spores of this organism usually fail to germinate.

The large volatilization of ammonia during the "sweating out process" appears to be an important factor for killing spores of *P. microspora*. The presence of alkaline salts in the compost favors a greater evolution of ammonia. In the attempt by the writer to kill the spores of this fungus by numerous fumigants, such as sulphur dioxide, formaldehyde, chloropicrin, dichloronitroethane, carbon disulphide, etc., only ammonia was found of value. It is necessary, therefore, to prepare synthetic composts with somewhat more nitrogen than is necessary to produce the largest yields in order to insure a sufficient volatilization of ammonia. It is also necessary to add sufficient alkaline salts and adjust the pH at 8.0 to 8.5, not only to assist the evolution of ammonia, but also to build up a high buffering capacity of the compost for reasons previously described. This condition of the compost, however, is highly favorable to the Coprinus sp. It is thus essential to strike a balance: make the conditions in the compost somewhat more favorable to Coprinus than to *P. microspora*, since the former is less dangerous to the mushroom crop. The mushroom mycelium can ultimately grow over the mycelial growth of the Coprinus sp. that preceded it; but when *P. microspora* follows the growth of the mushroom mycelium, the crop is greatly reduced or lost.

Summary

A method is described for preparing synthetic compost for mushroom culture from various source materials of N–P–K to produce about 13 lb. N, 4 lb. P_2O_5, and 10 lb. K_2O in a ton of fibrous material having 70 per cent. moisture, after allowing for the quantities of these three constituents present in the fibrous material. Several thousand tons of synthetic composts have been prepared by this method; it has been tested over a period of 5 years; and the yield has been as good as or better than that from horse manure composts.

One of the chief inadequacies of previous attempts to prepare satisfactory composts or "artificial manures" has been the omission of potash. By the addition of K_2O to synthetic composts prepared from spent licorice roots and brewers' grains, the yield was almost double that of similar composts to which only N and P_2O_5 were added.

The effect of potash, various sources of nitrogen, the subdivision and the character of the fibrous materials, the duration of and conditions for composting, the quantity of compost in the mushroom bed, and the relation of pH to the control of weed and disease fungi on the yield of mushrooms is reported.

A process is described in which tannin and lignin extracts are combined

with nitrogenous materials in order to avoid the lengthy outdoor composting period and to produce greater yields of mushrooms.

The writer is grateful for the cooperation of Mr. L. F. Lambert, Mr. Jacob M. Green, and Mr. Harry L. Hoffman. The writer is greatly indebted to the many companies which furnished by-products; especially, Dr. Houseman and Mr. Spiegel of McAndrews-Forbes Co.

L. F. Lambert Mushroom Spawn and Products
Coatesville, Pennsylvania

LITERATURE CITED

1. Friese, W. Über die Mineral Bestandteile von Pilzen. Zeit. Unters. Lebensmittel **57**: 604–613. 1929.
2. Hein, I. Straw composts for mushroom culture. Mycologia **22**: 39–42. 1930.
3. Lambert, E. B. Normal mushrooms from artificial manure. Science n.s. **70**: 126–128. 1929.
4. ————. Principles and problems of mushroom culture. Botanical Rev. **4**: 397–426. 1938.
5. Richards, E. H., and Hutchinson, H. B. Manufacture of nitrogenous fertilizers and the utilization of nitrogen-bearing solutions. U. S. Patent 1,471,979. 1923.
6. ————, and ————. Manufacture of nitrogenous fertilizers. U. S. Patent 1,619,679. 1927.
7. Sinden, J. W. Synthetic composts for mushroom growing. Pennsylvania Agr. Exp. Sta. Bull. 365. 1938.
8. Stoller, B. B. Thesis. Iowa State College. 1936.
9. ————, Smith, F. B., and Brown, P. E. A mechanical apparatus for the rapid, high temperature microbial decomposition of fibrous, cellulose material in the preparation of composts for mushroom culture. Jour. Amer. Soc. Agron. **29**: 717–723. 1937.
10. ————. Synthetic composts for mushroom culture. U. S. Patent 2,260,201. 1941.
11. ————. Oxidations by mushroom enzymes studied by cultural methods, and the relation of these oxidations to a mechanism for resisting disease. (Unpublished.)
12. Waksman, S. A., and Iyer, K. R. N. Synthesis of a humus-nucleus. Soil Sci. **34**: 43–70, 71–80. 1932.
13. ————, and McGrath, J. M. Preliminary study of chemical processes involved in the decomposition of manure by *Agaricus campestris*. Amer. Jour. Bot. **18**: 573–581. 1931.
14. ————, and Nissen, W. On the nutrition of the cultivated mushroom, *Agaricus campestris*, and the chemical changes brought about by this organism in the manure compost. Amer. Jour. Bot. **19**: 514–537. 1932.
15. ————, and Reneger, C. A. Artificial manure for mushroom production. Mycologia **26**: 38–45. 1934.

EFFECT OF BORON IN THE SUBSTRATE ON THE RATE OF NITRATE ABSORPTION AND ON NITROGEN DISTRIBUTION IN NASTURTIUM[1]

GEORGE B. BRIGGS

(WITH ONE FIGURE)

Boron was not considered indispensable for the normal growth and development of higher plants prior to 1923. In this year WARINGTON (**32**) proved conclusively that boron was essential for the growth and normal development of *Vicia faba* and several other members of the Leguminoseae. Subsequent investigators, JOHNSTON and DORE (**8, 9**), MCHARGUE and CALFEE (**11, 12**), MCMURTREY (**13**), SHIVE (**24**) and others have shown that a small continuous supply of boron is essential for the vegetative growth and reproduction of higher green plants, that the effective range of concentration varies with the species and environmental conditions, and that a deficiency of boron produces characteristic abnormal anatomical and cytological changes within the plant.

The purpose of the experiments here reported was to determine the effect of boron in the substrate on the rate of nitrate absorption and on the distribution of nitrogen in plant tissues.

Golden gleam nasturtium (*Tropaeolum majus*, var. florepleno) was selected for these investigations because it was found in preliminary experiments that this plant responded very rapidly to a deficiency of boron in the substrate (fig. 1).

Methods

Seed of uniform size was selected, disinfected with 1:1000 mercuric chloride for five minutes, thoroughly rinsed in distilled water, and placed between clean moist blotters. When the primary root was 3 cm. long the germinating seeds were placed on paraffined nets stretched over a complete nutrient solution after the method of SHIVE and ROBBINS (**25**). When the seedling had three leaves, uniform plants were transferred to paraffined cork stoppers (**25**) which were placed in holes in paraffined tin tops of one-gallon glass candy jars. These culture vessels contained a complete nutrient solution. The plants were continuously aerated and continuously supplied with nutrient solution by the method of SHIVE and STAHL (**26**). A three-salt stock solution of the following composition was used: KH_2PO_4, 0.0021 M; $Ca(NO_3)_2$, 0.0042 M; and $MgSO_4$, 0.0021 M.

In the complete solution boron, manganese, and zinc were also supplied as boric acid, manganese sulphate, and zinc sulphate, respectively, at the rate of 0.25 p.p.m. of solution. Iron was added as ferric tartrate at the rate of 1 p.p.m. Enough sulphuric acid was added to make the initial pH of the nutrient solution 4.0 to 4.2.

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Physiology.

The method of determining absorption rates was essentially that outlined by CLARK and SHIVE (4). At the end of the absorption interval of nine hours the plants were separated into leaf-blades, petioles, stems, and roots and the weight recorded. Representative aliquots of the tissue fractions, placed in trays, were carefully cut into small pieces and dried at 70° C. in an oven through which air was rapidly circulating. Dry weights of the fractions were then determined and the tissue stored in air tight bottles. The solution in which these plants had been growing and the rinsings of the necessary supplementary vessels were collected quantitatively and made to volume. Nitrogen determinations were made on aliquots of this solution in order to determine the amount of nitrogen remaining in the solution. Since a known amount of nitrogen was added, the difference between these two

FIG. 1. Characteristic appearance of boron deficient (left) and of normal nasturtium shoot.

determinations was the amount absorbed by the plants during the nine hour test period.

Two series of nasturtium plants were used in these experiments. Series I consisted of thirty-six-day-old plants; series II of fifty-day-old plants. Previous to the time of selection the plants in both series had received a complete nutrient solution continuously. This solution was identical with the solution in which the normal plants were grown during the absorption studies.

In series I thirty-six-day-old plants were used to study the effect of a change of boron concentration in the growth media on the rates of nitrate absorption and nitrogen distribution in young nasturtiums over a relatively short period of time, fourteen days. Absorption tests were made when these plants had been on their respective boron treatments for 4, 6, 8, 11, and 14 days.

Fifty-day-old plants were used in series II to study the effect of a change of boron concentration in the growth media on the rates of nitrate absorption and nitrogen distribution in relatively mature nasturtiums over an extended period of time, thirty-one days. Absorption studies were made after the plants had been on their respective boron treatments for 7, 13, 19, 26, and 31 days.

When the nasturtium plants were selected for the absorption tests, one-third were placed in the nutrient solution to which no boron was added (group A); one-third continued to receive the solution containing one-quarter of a part per million of boron (group B); and the remaining one-third were placed in the nutrient solution containing a toxic concentration of boron, fifteen parts per million (group C). Twelve cultures, each containing three plants, were grown at each boron level.

A reduced iron micro-Kjeldahl method was used for the total, and total soluble nitrogen determinations. After the nitrogen was reduced (**14**), two-tenths of a gram of sodium sulphate was added by means of a dry funnel and 1 ml. of concentrated sulphuric acid containing 12 mg. of selenium oxychloride per ml. was pipetted into the test tube. The sample was then digested and subsequently distilled and titrated.

Dry, finely ground tissue was analyzed for ammonium nitrogen according to the method of PUCHER, VICKERY, and LEAVENWORTH (**20**).

Nitrate nitrogen determinations were made by using the phenoldisulphonic acid method described by SNELL and SNELL (**27**).

Results and discussion

ABSORPTION STUDIES

BORON DEFICIENCY.—The data in table I show the milligrams of nitrate nitrogen absorbed in nine hours by nasturtium plants of series I grown at three levels of boron for 4, 6, 8, 11, and 14 days.

It will be observed from a study of this table that a deficiency of boron in the nutrient solution has no immediate effect on the rate of absorption of nitrate nitrogen. Plants grown in boron deficient solutions for more than six days showed a progressive decrease in nitrate absorption, compared with normal plants, with the length of time on treatment. While the total nitrate nitrogen absorbed per culture by boron deficient plants decreased slightly with the length of time of treatment, the total nitrate nitrogen absorbed per culture by normal plants increased very markedly.

The decrease in rate of nitrate absorption by nasturtium plants grown in solutions to which no boron was added increased with the severity of the deficiency symptoms. The external appearance of the tops of nasturtium plants which had been growing for four days in minus-boron solutions were quite normal; the roots, however, were light gray in color compared with the silvery-white color of the normal roots. At the end of the sixth day very definite deficiency symptoms were evident; the terminal growing point of the stem was not developing so rapidly and was a darker green than that of

TABLE I

AVERAGE GREEN AND DRY WEIGHTS AND TOTAL AND AVERAGE RATES OF NITRATE NITROGEN ABSORPTION OF THIRTY-SIX-DAY-OLD NASTURTIUMS OF SERIES I AS INFLUENCED BY BORON CONCENTRATION OF THE SUBSTRATE

DAYS ON TREATMENT	AVERAGE GREEN WEIGHT PER PLANT			AVERAGE DRY WEIGHT PER PLANT			TOTAL NO_3–N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS			NO_3–N ABSORBED PER GRAM DRY WEIGHT IN 9 HOURS		
	P.P.M. BORON			P.P.M. BORON			P.P.M. BORON			P.P.M. BORON		
	0.0	0.25	15.0	0.0	0.25	15.0	0.0	0.25	15.0	0.0	0.25	15.0
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
4	6.60	7.30	7.10	0.72	0.73	0.73	14.69	15.34	14.91	6.84	7.11	6.84
6	7.32	8.56	8.86	0.89	0.84	1.01	12.28	15.68	16.97	4.59	6.21	5.58
8	9.26	12.35	10.25	1.18	1.17	1.12	12.74	20.01	16.93	3.60	5.67	5.04
11	9.43	24.00	16.50	1.27	2.22	1.64	11.24	33.70	21.84	2.97	5.04	4.41
14	13.95	34.10	22.50	2.03	3.11	2.19	11.63	38.35	23.00	1.89	4.14	3.51

comparable normal plants. The roots showed definite signs of injury; the large roots were not increasing in length, and the terminal meristems were much darker in color than those of normal roots. In contrast with the long, lateral branch roots characteristic of the normal root system, numerous short, weak laterals developed behind the injured terminal meristems. This lack of development of additional root surface in boron deficient plants and the progressive increase in the extent of the root system of normal plants was reflected in the total absorption per culture during the nine-hour absorption interval. It will be observed from table II that nasturtium plants which were grown for six days in solutions without boron absorbed approximately the same total amount of nitrogen during the nine-hour absorption interval as did plants grown in similar solutions for fourteen days. On the other hand, the total nitrogen absorbed per culture per nine hours by normal plants increased progressively during the period of the absorption studies.

TABLE II

EFFECT OF BORON ON FRESH WEIGHT OF ROOTS AND TOTAL NITRATE NITROGEN ABSORBED BY NASTURTIUMS TRANSFERRED TO BORON TREATMENTS WHEN 36 DAYS OLD, SERIES I

DAYS ON BORON TREATMENT	TOTAL FRESH WEIGHT OF ROOTS PER CULTURE		TOTAL NO_3–N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS	
	P.P.M. BORON		P.P.M. BORON	
	0.0	0.25	0.0	0.25
	gm.	*gm.*	*mg.*	*mg.*
4	3.89	5.73	14.69	15.34
6	4.23	8.10	12.28	15.68
8	5.02	12.02	12.74	20.01
11	4.48	20.20	11.24	33.70
14	6.52	28.50	11.63	38.35

The absorption data for the second series of nasturtium plants are presented in table III. These plants were fifty days old when absorption tests were started and, although they were not growing as rapidly (indicated by the increase in rate of absorption) as the younger plants of the first series, the absorption trends are the same. Deficiency symptoms had appeared in the roots and tops of minus-boron plants at the time the first absorption tests were made. The terminal root meristems of most of the main roots of nasturtium plants which were grown in nutrient solutions without boron for more than eight days were inactive. They soon acquired the characteristic "club-like" appearance as the result of the death of numerous small lateral meristems which in many cases never completely penetrated the cortex and epidermis. The roots of deficient plants were stunted and thicker than normal roots and were a dark gray in color.

Although an absence of boron from the nutrient solution has no immediate effect on the rate of nitrate absorption by nasturtium plants, the growth of normal roots placed in solutions containing no added boron was quickly arrested. There seems to be a close correlation between the subsequent de-

TABLE III

AVERAGE GREEN AND DRY WEIGHTS AND TOTAL AND AVERAGE RATES OF NITRATE NITROGEN ABSORPTION BY FIFTY-DAY-OLD NASTURTIUMS OF SERIES II AS INFLUENCED BY BORON CONCENTRATION OF THE SUBSTRATE

DAYS ON TREATMENT	AVERAGE GREEN WEIGHT PER PLANT			AVERAGE DRY WEIGHT PER PLANT			TOTAL NO_3–N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS			NO_3–N ABSORBED PER GRAM DRY WEIGHT IN 9 HOURS		
	P.P.M. BORON			P.P.M. BORON			P.P.M. BORON			P.P.M. BORON		
	0.0	0.25	15.0	0.0	0.25	15.0	54.44	54.44	54.44	2.07	2.07	2.07
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
0	102.9	102.9	102.9	8.6	8.6	8.6	0.0	0.25	15.0	0.0	0.25	15.0
7	110.8	99.0	100.3	10.8	8.8	9.5	46.33	51.48	49.88	1.44	1.98	1.71
13	140.0	133.3	134.7	17.1	12.7	14.0	43.09	59.82	54.18	0.81	1.53	1.26
19	151.5	165.0	175.2	19.9	14.5	16.8	44.78	65.84	52.92	0.72	1.53	1.08
26	145.0	215.0	189.8	20.0	19.7	19.1	29.40	57.92	49.28	0.45	0.99	0.90
31	123.3	249.4	204.8	16.2*	24.7	24.3	20.41	82.25	62.69	0.45	1.08	0.90

* These plants were slightly inferior at start of experiment.

crease in rate of nitrate absorption and the extent of the root injury since the first measurable deviation from the normal rate of absorption was coincident with the first definite external symptoms of injury to the roots.

Numerous workers (**21, 24, 28, 29, 30, 33**) have recorded the anatomical changes in cells and tissues of the roots of various boron-deficient plants. In all of the plants investigated the meristematic tissues were the first to show the effects of the absence of boron. An early modification of regular mitotic divisions occurs with consequent pathological tissue changes.

Since a deficiency of boron is first evident in those regions of the root in which rapid cytological and histological changes are taking place, it might be logical to assume that the decrease in rate of absorption is a result of the impaired physiological activity of these tissues brought about by an inadequate supply of boron.

PREVOT and STEWARD (**19**) have presented evidence which indicates that those cells which are capable of most active growth attain the greatest degree of salt accumulation. In the normal root these are the cells of the apical segment, the same region which in boron-deficient plants is so completely disorganized.

BORON TOXICITY.—In preliminary experiments it was found that a concentration of 15 parts per million of boron in the substrate produced toxicity symptoms in nasturtiums.

It will be observed from tables II and III that a toxic concentration of boron in the nutrient solution has no immediate effect on the rate of nitrate absorption; and that when plants are grown in solutions containing a toxic concentration of boron, it accounts for the decrease in the rate of nitrate nitrogen absorption.

EFFECT OF BORON ON GREEN AND DRY WEIGHTS AND MOISTURE CONTENT

It will be observed from table I that, although there was a progressive increase in the average green weight of the plants grown at the three levels of boron during the experimental period of fourteen days, at the end of this time the plants receiving a quarter of a part per million of boron were far superior to those plants grown in deficient and toxic concentrations of boron. When this growth criterion was used, the boron deficient plants were inferior not only to the normal plants but also to the plants grown in solutions containing a toxic concentration of boron. The boron deficient plants made only two-fifths the growth, on the green weight basis, of normal plants. The nasturtiums grown in solutions containing fifteen parts per million of boron made only two-thirds the growth of comparable normal plants during the fourteen days on treatment.

The difference observed in the growth response of boron deficient plants, as measured by the average dry weight per plant, was not as marked as was the case when green weight was used as the growth criterion. The relatively low moisture content of the boron deficient plants (table IV) accounts

for the partial masking of the differences between boron treatments when average dry weight per plant was used as the measurement of growth.

Table III also shows the average green and dry weights per plant of the plants of series II. It is observed from these data that nasturtiums grown in nutrient solutions without added boron for nineteen days were only slightly smaller, on the green weight basis, than comparable normal plants; but on the same basis (green weight production), the plants grown for thirty-one days in nutrient solutions to which no boron was added were much smaller than control plants. The nasturtiums grown in solutions containing fifteen parts per million of boron were only slightly inferior to comparable normal plants at the end of the experimental period (31 days), when average green weight was used as the growth criterion.

TABLE IV

MOISTURE CONTENT OF NASTURTIUMS AS EFFECTED BY THE BORON CONCENTRATION OF THE SUBSTRATE

SERIES I					SERIES II				
DAYS ON TREATMENT	AGE OF PLANTS	PERCENTAGE MOISTURE			DAYS ON TREATMENT	AGE OF PLANTS	PERCENTAGE MOISTURE		
		P.P.M. BORON					P.P.M. BORON		
		0.0	0.25	15.0			0.0	0.25	15.0
	days	%	%	%		*days*	%	%	%
4	40	89.1	90.0	89.8	0	50	91.7	91.7	91.7
6	42	87.9	90.2	88.6	7	57	90.5	90.6	90.6
8	44	87.3	90.6	89.1	13	63	88.1	89.9	89.5
11	47	86.5	90.7	90.0	19	69	87.4	90.6	90.6
14	50	85.4	90.8	90.3	26	76	86.5	90.8	89.9
					31	81	86.8	90.3	88.2

The low moisture content of the boron deficient plants would account for the slight difference between the normal and boron deficient plants of series II, when average dry weight is used as the growth criterion. There was relatively little difference in the average green and dry weights of nasturtiums grown in solutions containing 0.25 and 15 p.p.m. of boron.

A concentration of 15 p.p.m. of boron in the substrate had a more pronounced effect on the growth of young nasturtiums than on mature plants when average green weights were compared (tables I, III). It is possible that boron accumulated in the leaves of the larger plants to such an extent that the active soluble fraction although still in excess of normal boron concentration was relatively low in the actively synthesizing regions of the mature plants; in the young plants with high absorption rates and a predominance of actively synthesizing cells the soluble boron content was relatively high.

The decrease in percentage moisture and corresponding increase in percentage dry weight, in terms of green weight (of boron deficient plants), would indicate less succulence, less metabolic activity, and the lowest growth

rate in boron deficient nasturtiums in comparison with those grown in solutions to which boron was added. ELTINGE (**6**) reports in this connection that boron deficient *Zea mays* plants had a higher percentage dry weight than control plants.

SCHMUCKER (**22**) has suggested that boron exerts some control over the swelling of plasma colloids, at least in actively growing cells. He found that the pollen tubes of certain tropical water lilies (Nymphaea sp.) burst soon after forming in a sugar solution of the same concentration as the nectar on the stigma of the species under investigation. In the stigmatic fluid, of course, they developed normally. He found that the addition of 0.001 to 0.01 per cent. boric acid prevented the bursting of the pollen tubes in the artificial media. In connection with SCHMUCKER's work it is interesting to note that BOBKO, MATVEEVA, and SYVOROTKIN (**2**), and BERTRAND

TABLE V

MILLIGRAMS OF INORGANIC NITROGEN PER GRAM OF DRY WEIGHT OF 50-DAY-OLD NASTURTIUMS (SERIES I) GROWN FOR 14 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTION CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN

PLANT FRACTIONS AND TREATMENT	INORGANIC NITROGEN		
	NH_4	NO_3	TOTAL
	mg./gm.	*mg./gm.*	*mg./gm.*
Leaf blades			
0.0 p.p.m. boron	0.0041	None	0.0041
0.25 p.p.m. boron	0.0039	0.0765	0.0804
15.0 p.p.m. boron	0.0033	0.1640	0.1673
Stems			
0.0 p.p.m. boron	0.0086	0.1173	0.1259
0.25 p.p.m. boron	0.0072	0.8700	0.8772
15.0 p.p.m. boron	0.0023	0.6130	0.6153
Roots			
0.0 p.p.m. boron	0.0351	0.3720	0.4071
0.25 p.p.m. boron	0.0819	1.0600	1.1420
15.0 p.p.m. boron	0.0364	0.6620	0.6984

and SILBERSTEIN (**1**) found an accumulation of boron in the flowers of apple and *Lilium candidum*, respectively, and that the highest concentrations were found in the stigmas of both flowers.

WARINGTON (**33**), and SOMMER and SOROKIN (**28**) have presented some additional evidence which seems to indicate that an initial effect of boron deficiency is a change in the water relations of meristematic cells, as indicated by hypertrophy of cambium cells and periblem tissue of roots. These observations were made on actively growing individual cells and were associated with the first microscopically visible changes resulting from a deficiency of boron.

The data in table IV show that there was a progressive decrease in the percentage of moisture in boron deficient nasturtiums with length of time of treatment, and it is evident that there was a decrease in growth (average green weight per plant) in comparison with normal plants (tables I, III).

These results do not substantiate or disprove SCHMUCKER's suggestion because he was observing the initial changes in individual cells and not the ultimate pathological effects on plant tissues. Boron is not the only element whose absence from the growth medium results in a decreased moisture content; a deficiency of calcium (**18**) or nitrogen (**10, 15, 16**) will produce similar results.

KRAUS and KRAYBILL (**10**) observed that, in general, there was a close correlation between total nitrogen, nitrate nitrogen, and moisture; and that plants low in total nitrogen and nitrate nitrogen had a low moisture content. They suggested that a rapid growth rate which resulted in the formation of thin walled cells, and an increased percentage of amphoteric

TABLE VI

INORGANIC NITROGEN IN 81-DAY-OLD NASTURTIUMS OF SERIES II GROWN FOR 31 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN, EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

PLANT FRACTIONS AND TREATMENT	INORGANIC NITROGEN		
	NH_4	NO_3	TOTAL
	mg./gm.	*mg./gm.*	*mg./gm.*
Leaf blades			
0.25 p.p.m. boron*	0.0045	None	0.0045
0.0 p.p.m. boron	0.0057	None	0.0057
0.25 p.p.m. boron	0.0043	None	0.0043
15.0 p.p.m. boron	0.0036	None	0.0036
Stems			
0.25 p.p.m. boron*	0.0061	0.561	0.5671
0.0 p.p.m. boron	0.0297	0.181	0.2107
0.25 p.p.m. boron	0.0094	0.426	0.4354
15.0 p.p.m. boron	0.0069	0.078	0.0849
Roots			
0.25 p.p.m. boron*	0.0341	1.550	1.5841
0.0 p.p.m. boron	0.0505	0.597	0.6475
0.25 p.p.m. boron	0.0508	0.966	1.0168
15.0 p.p.m. boron	0.0264	0.427	0.4534

* Analyses made at beginning of experiment when plants were 50 days old.

substances was among the factors which would account for a relatively high moisture content. Since boron-deficient nasturtiums were low in total nitrogen and nitrate nitrogen (tables V, VI), and since a deficiency of boron resulted in a decreased growth rate and the formation of fewer, thin walled cells, the percentage of amphoteric substances whose water holding capacity is relatively large was considerably less than in the rapidly growing vegetative tissues of comparable normal plants. This would indicate that the lower moisture content of boron deficient nasturtiums in series I and II, in comparison with normal plants, was not the direct result of a deficiency of boron itself; it was the result of a change in the nitrogen metabolism and absorption of boron deficient plants associated with an inadequate supply of this element.

TABLE VII

DISTRIBUTION OF NITROGENOUS FRACTIONS IN 50-DAY-OLD NASTURTIUMS OF SERIES I GROWN FOR 14 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

Plant fractions and treatments	Total inorganic		Soluble organic		Total soluble		Insoluble organic		Total organic nitrogen	Total nitrogen
	mg./gm.	%*	*mg./gm.*	%*	*mg./gm.*	%*	*mg./gm.*	%*	*mg./gm.*	*mg./gm.*
Leaf blades										
0.0 p.p.m. boron	0.0041	0.1	0.816	23.4	0.82	23.5	2.67	76.6	3.486	3.49
0.25 p.p.m. boron	0.0804	1.6	1.170	23.5	1.25	25.1	3.72	74.9	4.891	4.97
15.0 p.p.m. boron	0.1673	3.6	1.133	24.1	1.30	27.7	3.39	72.3	4.523	4.69
Stems										
0.0 p.p.m. boron	0.1259	5.1	1.244	50.0	1.37	55.1	1.11	44.9	2.354	2.48
0.25 p.p.m. boron	0.8772	25.4	1.153	33.4	2.03	58.8	1.42	41.2	2.573	3.45
15.0 p.p.m. boron	0.6153	21.3	0.955	33.1	1.57	54.4	1.31	45.6	2.265	2.88
Roots										
0.0 p.p.m. boron	0.4071	11.9	0.743	21.7	1.15	33.6	2.28	66.4	3.023	3.43
0.25 p.p.m. boron	1.1419	21.6	1.128	21.4	2.27	43.0	3.01	57.0	4.138	5.28
15.0 p.p.m. boron	0.6984	14.7	0.922	19.4	1.62	34.1	3.13	65.9	4.052	4.75

* Percentage of total nitrogen.

The effect of boron in the substrate on the distribution of nitrogen in nasturtium

The experimental data presented in this section represent the analysis of the tissues of fifty-day-old (series I) and eighty-one-day-old (series II) nasturtium plants grown for fourteen and thirty-one days, respectively, in nutrient solutions containing a deficient (0.0 p.p.m. B), a normal (0.25 p.p.m. B) and a toxic (15.0 p.p.m. B) concentration of boron.

Boron deficiency.—Experimental evidence has shown that in nasturtium plants here employed as well as in other boron-deficient plants carbohydrates accumulate (**9, 24, 29, 30, 31**) and the regions of meristematic activity (**3, 7, 28, 33**) are the first to show symptoms of an inadequate supply of boron. Since rapid protoplasm formation is associated with these regions of active cell division, it appears logical to assume that the pathological phenomena accompanying boron deficiency might very well be associated with a disturbance in the nitrogen metabolism. The analytical data of tables VII and VIII tend to substantiate this assumption.

One of the principal uses of carbohydrates is in protein synthesis. Therefore, if there is little or no external supply of nitrogen (**10, 15, 16**) or if there is a decrease in the rate of synthesis in all or some forms of organic nitrogen (**17**), carbohydrates will tend to accumulate. Although it is evident from the absorption experiments (tables I, III) that boron deficient nasturtiums were very much limited in their ability to absorb nitrates, it must be remembered that the initial decrease in the rate of absorption of plants growing in solutions with no added boron was coincident with the appearance of visible injury to the roots.

A study of table V shows that ammonium accumulated in the leaves of fifty-day-old nasturtiums (series I), which had been growing for fourteen days in minus-boron solutions. In fact, ammonium was the only form of inorganic nitrogen found in the leaves of boron deficient nasturtiums. In normal leaves, on the other hand, ninety-five per cent. of the inorganic nitrogen was nitrate. This might indicate that nitrate was being reduced in the boron deficient nasturtium leaf blades; this reduced nitrogen, however, was not being fully utilized even in the early stages of nitrogen assimilation.

The content of organic nitrogen, both soluble and insoluble, expressed as the percentage of total organic nitrogen, was the same in the leaves of the boron deficient and normal plants; the actual amount in each case, however, was less in the boron deficient plants (table VII). This suggests a lower rate of organic nitrogen synthesis in the boron deficient leaf blades than in those of the control plants.

Ammonium also accumulated in the stems of boron deficient plants although the total inorganic nitrogen content was very low in comparison with normal plants. There was an accumulation of soluble organic nitrogen in the stems of boron deficient plants. There was no appreciable difference in the ammonium content of boron deficient and normal plants but there was an increase in the soluble nitrogen content.

TABLE VIII

DISTRIBUTION OF NITROGENOUS FRACTIONS IN 81-DAY-OLD NASTURTIUMS OF SERIES II GROWN FOR 31 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

PLANT FRACTIONS AND TREATMENTS	TOTAL INORGANIC		SOLUBLE ORGANIC		TOTAL SOLUBLE		INSOLUBLE ORGANIC		TOTAL ORGANIC NITROGEN	TOTAL NITROGEN
	mg./gm.	%†	*mg./gm.*	%†	*mg./gm.*	%†	*mg./gm.*	%†	*mg./gm.*	*mg./gm.*
Leaf blades										
0.25 p.p.m. boron*	0.0045	0.10	0.916	21.3	0.92	21.4	3.39	78.6	4.30	4.31
0.0 p.p.m. boron	0.0057	0.26	0.854	39.0	0.86	39.2	1.33	60.8	2.18	2.19
0.25 p.p.m. boron	0.0043	0.10	1.026	23.7	1.03	23.8	3.31	76.2	4.33	4.34
15.0 p.p.m. boron	0.0036	0.10	0.976	26.3	0.98	26.4	2.73	73.6	3.70	3.71
Stems										
0.25 p.p.m. boron*	0.5671	20.10	1.293	46.1	1.86	66.2	0.95	33.8	2.24	2.81
0.0 p.p.m. boron	0.2107	7.00	1.739	58.0	1.95	65.0	1.05	35.0	2.79	3.00
0.25 p.p.m. boron	0.4354	17.21	1.355	53.5	1.79	70.7	0.74	29.3	2.10	2.53
15.0 p.p.m. boron	0.0849	3.84	1.295	58.7	1.38	62.5	0.83	37.5	2.13	2.21
Roots										
0.25 p.p.m. boron*	1.5841	31.06	0.896	17.6	2.48	48.6	2.62	51.4	3.52	5.10
0.0 p.p.m. boron	0.6475	17.90	1.202	33.4	1.85	51.3	1.76	48.7	2.96	3.61
0.25 p.p.m. boron	1.0168	23.21	1.073	24.5	2.09	47.7	2.29	52.3	3.36	4.38
15.0 p.p.m. boron	0.4534	11.99	0.717	18.9	1.17	30.9	2.61	69.1	3.33	3.78

* Analyses made at beginning of experiment when plants were 50 days old.
† Percentage of total nitrogen.

The accumulation of soluble organic nitrogenous compounds coupled with a disturbance in the insoluble organic nitrogen indicates an interruption at some stage in the intermediate synthetic processes before protein formation in boron deficient nasturtiums. Since, however, there was an accumulation of ammonium and carbohydrates, the relatively high content of soluble organic nitrogen in the stems, as compared with check plants, might also indicate proteolysis.

On the basis of analyses of leaf blades and stems of the plants of series I (table VII) it appears that without an adequate supply of boron the synthesis of organic nitrogenous compounds is retarded in the initial stages of the nitrogen assimilatory processes. The analyses of the root tissues do not confirm this.

The distribution of nitrogen in eighty-one-day-old nasturtium plants showing boron deficiency symptoms (table VIII) was essentially the same as in the fifty-day-old boron deficient plants. In all the organs of the boron deficient nasturtium plants of both series except the roots of series I there was an accumulation of ammonium when the boron deficient plants were compared with normal plants on the basis of their ammonium content. The mature plants of series II which were grown for thirty-one days in solutions without added boron showed a higher content of soluble organic nitrogen in the stems and roots and in the leaf blades on a relative basis than in similar organs of control plants. The protein nitrogen was low in the leaf blades and roots of boron deficient nasturtiums in comparison with normal plants. The accumulation of soluble organic nitrogen compounds associated with boron deficiency may be the result of a low rate of protein synthesis. Since, however, this distribution of the organic nitrogenous compounds is more characteristic of the mature nasturtiums of series II, which were grown for an extended period of time (31 days) in solutions without added boron, it is quite possible that the change from normal nitrogen distribution was due to proteolysis.

SHIVE (**24**) and WADLEIGH and SHIVE (**31**) have shown, as the result of extensive microchemical studies on boron deficient cotton seedlings, that scattered cells in the tissues of the stem tips became more acid than comparable normal cells as deficiency symptoms increased in severity. Although no ammonium nitrogen was supplied to these plants, ammonium nitrogen accumulated, especially in the more acid cells. They also reported an accumulation of carbohydrates and a progressive degeneracy of the protoplasm as the disease symptoms became more pronounced. JOHNSTON and DORE (**9**) have presented data which indicate that the accumulation of carbohydrates, especially in the leaves of boron deficient tomato plants, occurs because of injury to the conducting systems. VAN SCHREVEN (**29, 30**) also suggested that the partial blocking of the conducting systems in tobacco and tomato resulting from injury due to boron deficiency impeded the translocation of starch and sugars. In studying the anatomical changes resulting from boron deficiency in young sugar beet plants, ROWE (**21**) observed that

callose (callus) plugs occurred much more frequently in the phloem of diseased plants than in the sieve tubes of healthy beets; and that they were sometimes frequent in strands of boron deficient plants which did not exhibit any other abnormalities. She suggests that their development may be the characteristic precursor of the subsequent degeneration in the mature tissue of vascular strands.

The accumulation of both ammonium and carbohydrates and the failure of the cells of meristematic tissues to divide and differentiate normally, strongly suggests that boron is intimately associated with the initial processes of nitrogen assimilation. It is evident, therefore, on the basis of the data presented in this section, that the presence of boron is one of the essential factors in the utilization of (reduced) nitrogen in the assimilatory processes.

Boron toxicity.—The distribution of nitrogenous fractions in the tissues of the nasturtium plants of series I and II which showed boron toxicity symptoms are included in tables V, VI, VII, and VIII. These plants were grown in solutions containing 15 p.p.m. of boron.

It is observed from the data in table V that the leaf blades of nasturtiums of series I showing boron toxicity symptoms contained more than twice as much nitrate and less ammonium than did the leaves of comparable normal plants. This accumulation of nitrates suggests that nitrate was not being reduced as rapidly in the leaf blades showing boron toxicity symptoms as in the normal leaves. The fact that no nitrate was found in the leaf tissues of the plants of series II regardless of boron concentration does not necessarily refute this conclusion; these plants were older than the plants of series I and were harvested at a different time of year. Table VII shows that in series I there was a lower content of insoluble organic nitrogen and a higher content of soluble organic nitrogen in the boron toxic leaf blades than in those of control plants; a condition similar to comparable leaf blades of series II (table VIII). These deviations from the normal condition may be due to proteolysis since the leaves showed characteristic boron toxicity symptoms.

Further study of table VII shows that the total soluble nitrogen content of the stems and roots of the boron-toxic plants of series I was lower and that the insoluble organic nitrogen content of the stems and roots of the boron-toxic plants of series I was higher than in those of normal plants. This would suggest that a high concentration of boron accelerates the formation of complex nitrogenous compounds in the stems and roots of nasturtiums.

It is significant that the percentage distribution of the various nitrogenous fractions in the roots of the boron-toxic nasturtiums of series I and II was almost the same (tables VII, VIII). In both cases there was a lower content of total soluble nitrogen and a higher content of protein nitrogen than in control plants. Since root development was slightly retarded in boron toxic nasturtiums and the rate of nitrate absorption was lower than

in comparable normal plants (tables I, III), it is possible that there were fewer young active cells, and that the increase in insoluble organic nitrogen indicated an accumulation of storage protein (**15, 17**).

An examination of the data in tables VII and VIII shows that the total organic nitrogen content of the roots of both normal and boron-toxic plants in series I and of the stems and roots of both normal and boron-toxic plants in series II was nearly the same, but that the total organic nitrogen content of the leaf blades of boron-toxic nasturtiums of both series was lower than in normal nasturtiums. The leaf blades of series II showed the greatest external symptoms of injury due to boron. Although no boron determinations were made, it may be assumed from the work of EATON (**5**) and SCOFIELD and WILCOX (**23**), that the boron content of the leaf blades showing boron toxicity symptoms was relatively high. EATON found that those tissues which contained the highest concentration of boron manifested the greatest injury. WEBBER (**34**) has come to similar conclusions as the result of anatomical investigations.

It is evident, then, on the basis of the comparison of the data from tissue analysis just presented, that a toxic concentrataion of boron has a definite effect on the accumulation of protein nitrogen.

Summary

1. Nasturtiums grown in nutrient solutions to which no boron was added showed a progressive decrease in nitrate absorption, compared with normal plants, with the length of time of treatment. There was no close correlation between the subsequent decrease in rate of nitrate absorption and the extent of root injury.

2. Ammonium and carbohydrates as well as soluble organic nitrogen accumulated in nasturtiums showing boron deficiency symptoms. It appears, therefore, that in the absence of an adequate supply of boron the amination of carbohydrate derivatives is inhibited.

3. The proportion of total soluble to insoluble organic nitrogen was lower in the stems and roots of plants showing boron toxicity symptoms than in control plants. This suggests an accelerated synthesis of complex nitrogenous compounds.

NEW JERSEY AGRICULTURE EXPERIMENT STATION
RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

1. BERTRAND, G., and SILBERSTEIN, L. Sur la repartition du bore dans les organes du lis blanc. Compt. Rend. Acad. Sci. **206**: 796–799. 1938.
2. BOBKO, E. V., MATVEEVA, T. V., and SYVOROTKIN, G. S. Récherches sur le rôle du bore dans les plantes. Ann. Agron. **5**: 801–803. 1935.
3. BRENCHLEY, W. E., and THORNTON, H. A. The relation between the development, structure, and function of the nodules of *Vicia faba*,

as influenced by the presence or absence of boron in the nutrient medium. Proc. Roy. Soc. (London) **98**: 373–399. 1925.

4. CLARK, H. E., and SHIVE, J. W. The influence of the pH of a culture solution on the rates of absorption of ammonium nitrate nitrogen by the tomato plant. Soil Sci. **37**: 203–225. 1934.
5. EATON, F. M. Boron in soils and irrigation waters and its effect on plants with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bull. 448. 1935.
6. ELTINGE, E. T. Effect of boron deficiency upon the structure of *Zea mays*. Plant Physiol. **11**: 765–778. 1936.
7. HAAS, A. R. C., and KLOTZ, L. J. Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia **5**: 175–188. 1931.
8. JOHNSTON, E. S., and DORE, W. H. The relation of boron to the growth of the tomato plant. Science n.s. **67**: 324–325. 1928.
9. ————, and ————. The influence of boron on the chemical composition and growth of the tomato. Plant Physiol. **4**: 31–62. 1929.
10. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
11. MCHARGUE, J. S., and CALFEE, R. K. Effect of boron on the growth of lettuce. Plant Physiol. **7**: 154–161. 1932.
12. ————, and ————. Further evidence that boron is essential for the growth of lettuce. Plant Physiol. **8**: 305–315. 1932.
13. MCMURTREY, J. E. The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated solutions. Jour. Agr. Res. **38**: 371–380. 1929.
14. MOORE, R. H. A micro-Kjeldahl method including nitrates. Bot. Gaz. **100**: 250–252. 1938.
15. NIGHTINGALE, G. T. Nitrogen nutrition of green plants. Bot. Rev. **3**: 85–174. 1937.
16. ————, SCHERMERHORN, L. G., and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
17. ————, ————, and ————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
18. ————, ADDOMS, R. M., ————, and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
19. PREVOT, P., and STEWARD, F. C. Salient features of the root system relative to the problem of salt absorption. Plant Physiol. **11**: 509–534. 1936.

20. Pucher, G. W., Vickery, H. B., and Leavenworth, C. S. Determination of ammonia and of amide nitrogen in plant tissue. Ind. & Eng. Chem. Anal. Ed. **7**: 152–156. 1935.
21. Rowe, E. A. A study of heart-rot of young sugar-beet plants grown in culture solutions. Ann. Bot. **50**: 735–746. 1936.
22. Schmucker, T. Über den Einfluss von Borsäure auf Pflanzen, insbesondere keimende Pollenkörner. Planta **23**: 264–283. 1935.
23. Scofield, C. S., and Wilcox, L. V. Boron in irrigation waters. U. S. Dept. Agr. Tech. Bull. 264. 1931.
24. Shive, J. W. The adequacy of the boron and manganese content of natural nitrate of soda to support growth in sand culture. New Jersey Agr. Exp. Sta. Bull. 603. 1936.
25. ————, and Robbins, W. R. Methods of growing plants in solution and sand cultures. New Jersey Agr. Exp. Sta. Bull. 636. 1937.
26. ————, and Stahl, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**: 317–323. 1927.
27. Snell, F. D., and Snell, C. T. A colorimetric method of analysis. D. Van Nostrand Co. New York, 1936. Nitrates. pp. 629–634. 1936.
28. Sommer, A. L., and Sorokin, H. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant Physiol. **3**: 237–254. 1928.
29. Van Schreven, D. A. Uitwendige en enwendige symptomen van boriumgebrek bij tabak. Tijdschr. Plantenziek. **40**: 98–129. 1934. (English summary.)
30. ————. Uitwendige en enwendige symptomen van boriumgebrek bij tomaat. Tijdschr. Plantenziek. **41**: 1–26. 1935. (English summary.)
31. Wadleigh, C. H., and Shive, J. W. A microchemical study of the effects of boron deficiency in cotton seedlings. Soil Sci. **47**: 33–36. 1939.
32. Warington, K. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. **37**: 629–672. 1923.
33. ————. The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. **40**: 27–42. 1926.
34. Webber, I. E. Histological characteristics of plants grown in toxic concentrations of boron. Jour. Agr. Res. **50**: 189–194. 1935.

CHANGES IN CERTAIN WATER-SOLUBLE NITROGENOUS CONSTITUENTS OF BURLEY TOBACCO DURING CURING[1]

JAMES R. YOUNG AND R. N. JEFFREY

(WITH TWO FIGURES)

Introduction

An investigation of the chemical changes that take place in tobacco during curing is in progress at the Kentucky Agricultural Experiment Station. Attempts are being made to determine what the normal changes are and how they are influenced by such variables as temperature and relative humidity, which have been shown to influence the final quality of tobacco. A study of this type adds to the knowledge of leaf metabolism during starvation and drying. Knowledge of the chemistry of curing may lead to methods of producing high quality tobacco which have not been found empirically.

This paper reports the results of experiments on the changes that occur in certain water-soluble, nitrogenous constituents of the tobacco leaf during curing and of the effect of relative humidity upon these changes in chemical composition. Relative humidity was used as the variable rather than temperature because the work of JEFFREY (**3**) showed that differences in relative humidity cause larger differences in quality of the tobacco than do differences in temperature, within the limits encountered in practical Burley tobacco curing.

Chemical investigations of the tobacco plant have been made in many regions where tobacco is an important cash crop but very little of this work has dealt with the chemistry of curing. The most complete studies of the chemical changes that occur during the curing of tobacco were made by VICKERY (**10, 13**) and his associates at the Connecticut Agricultural Experiment Station. Primed leaves of a Connecticut shade-grown variety of cigar tobacco were cured in a curing shed and analyses were made of the leaves at different stages of curing. The analyses included insoluble nitrogen, several fractions of the soluble nitrogen, ash, carbohydrates, and ether extract.

In 1914 GARNER (**2**), of the Bureau of Plant Industry, reported on some chemical studies of tobacco curing. Both primed and stalk-cured leaves of several Connecticut cigar varieties were analyzed before and after curing. Analyses included several carbohydrate fractions, several organic acid fractions, several soluble nitrogen fractions, ash, and protein nitrogen. Ash and dry matter were expressed as grams per 100 leaves. The other analyses were expressed as percentage of dry weight.

Basis for reporting results

Since both the total sample weight and the dry weight of the leaves change during curing, neither of these forms a satisfactory basis for express-

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

ing results. Some investigators have expressed their findings as weight of component in a given number of leaves. Because of the great variation in size of Burley tobacco leaves, even if taken from the same stalk position, it would be necessary to take a very large sample to make the sampling error negligible. Since it was planned to cure the tobacco in curing chambers under controlled conditions, such large samples could not be used.

VICKERY *et al.* (**10, 13**) working with primed tobacco expressed their results as weight of a component per unit weight of the fresh leaves. Their method could not be used in this study because the tobacco leaves were cured on the stalk. Some investigators have expressed their data as weight of a component per unit area of the fresh leaf. This method is the one used in the present paper as it is possible to estimate the area of tobacco leaves while they are still attached to the stalk.

Methods

DETERMINATION OF LEAF AREA

The length and width of each of the four largest undamaged leaves on each plant were measured to the nearest centimeter before the plants were cut and a tag was attached to each leaf indicating its dimensions. Just before the plants were cut for the curing chambers, the leaves to be used in determining the regression equation relating the product of the length and the width to the area were cut from the plants and the midribs removed. Different methods of determining the relationship between these dimensions and the leaf area were used in the two years in which the study was conducted. In 1939 the lamina (leaf minus midrib) was weighed and plugs of known area were removed by means of a cork borer and weighed. The area of the leaf was calculated by multiplying the weight of the leaf by the ratio of area to weight of the plugs. The constants in the regression equation were evaluated by the method of least squares on data from 42 leaves. The regression equation is:

$$y = \frac{0.76x}{1000} - 0.015$$

where x = the product of the length and width of the leaf expressed in centimeters, and y = the leaf area in square meters. The standard error of estimate σ and the coefficient of correlation r_{xy}, were found to be:

$$\sigma_e = 0.009 \text{ m}^2$$
$$r_{xy} = 0.96$$

Due to the difficulties involved in taking plugs representative of the lamina, a different method was used in 1940. The length and width of the leaves were measured as before but the area was determined by blue-printing the lamina after the midrib had been removed and determining the area of the blueprints by weighing them. The regression equation, standard error of estimate, and the coefficient of correlation found from the data obtained from 100 leaves are:

$$y = \frac{0.65x}{1000} + 0.006$$

$$\sigma_e = 0.007 \text{ m}^2$$

$$r_{xy} = 0.96$$

Though the constants of these two regression equations are of different magnitude, the lines which they represent cross near the average leaf area so that the results obtained with the two equations are not widely different. Each equation, however, was used in calculating the corresponding year's results.

WOLF and GROSS (**15**) studied structural responses induced by topping and suckering. They gave data for 50 tobacco leaves which included measurements of length, width, and area. From their data the following regression equation may be calculated:

$$y = \frac{0.63x}{1000} + 0.03$$

$$r_{xy} = 0.99$$

WOLF and GROSS worked with a variety of flue-cured tobacco. One would not expect the regression equation to be the same for different varieties but the magnitude of the coefficient of correlation indicates that there is a high degree of linear correlation between the area of the tobacco leaf and the product of its length and width.

SAMPLING

The analyses were made of leaves from plants of Kentucky Experiment Station Burley no. 16 tobacco grown on the Station farm during the seasons of 1939 and 1940. In 1939 the plants were cut and speared on August 30, but were piled in the field until the next day to wilt. A fresh leaf sample was taken at the time of cutting. The tobacco was housed in the air-conditioned chambers described by O'BANNON (**6**) and used by JEFFREY (**3**) in studies of the effect of different air conditions during curing upon the quality of cured tobacco. Since these studies showed that the relative humidity was more critical than the temperature in controlling the quality of the cured tobacco, three different levels of relative humidity were used: one at about the optimum (70 per cent.); one above (78 per cent.); and one below (59 per cent.). The same temperature was maintained in all cases (75° F. or 23.9° C.).

The tagged leaves from the 6 plants on one stick constituted a sample. Since each tier supported 6 sticks it was possible to take one sample from each chamber at each of 6 different times during curing, in addition to the fresh-leaf sample. The time of sampling for each expressed as hours from cutting to sampling is recorded in the first column of table I.

When the samples were taken, the tagged leaves were examined and those damaged in housing were not included in the sample. After the tagged leaves were removed, the rest of the stick of tobacco was returned to the chamber in order to change the conditions surrounding the next sample as

TABLE I

CONCENTRATION OF CERTAIN CONSTITUENTS OF BURLEY TOBACCO LEAF WEB AT DIFFERENT TIMES DURING THE AIR-CURING PROCESS, 1939 CROP, STATED AS PERCENTAGE OF THE DRY WEIGHT AND AS WEIGHT PER SQUARE METER OF LEAF AREA

Time	Relative humidity	Dry matter	Sulphated ash		Total nitrogen		Nicotine nitrogen		Asparagine nitrogen		Glutamine nitrogen		Ammonia nitrogen		Amino acid N (by diff.)		Residual nitrogen	
hr.	%	*gm.*	%	*gm.*	%	*gm.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*gm.*
PFL	.....	39.6	20.8	8.2	4.5	1.78	0.62	246	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
0	.....	32.3	23.2	7.5	4.5	1.45	0.75	242	0.06	19	0.34	12	0.02	5	0.16	52	3.5	1.12
65	59	35.8	23.6	8.4	4.5	1.61	0.85	304	0.35	125	0.08	27	0.05	17	0.32	114	2.9	1.12
"	70	30.8	23.6	7.3	4.7	1.45	0.88	271	0.28	87	0.07	22	0.04	14	0.39	120	3.0	0.94
"	78	31.9	23.6	7.5	4.9	1.56	0.87	278	0.31	98	0.08	25	0.05	17	0.39	123	3.2	1.02
185	59	28.8	23.4	6.7	3.8	1.09	0.87	251	0.43	124	0.11	30	0.10	28	0.12	36	2.2	0.53
"	70	27.0	24.6	6.6	3.6	0.97	0.85	230	0.38	104	0.07	18	0.11	29	0.17	47	2.0	0.54
"	78	29.0	25.0	7.2	4.2	1.21	0.88	256	0.43	125	0.09	27	0.12	35	0.15	43	2.9	0.72
258	59	26.8	25.2	6.8	4.1	1.10	0.83	222	0.42	113	0.12	33	0.10	26	0.16	43	2.5	0.66
"	70	27.0	23.8	6.4	4.0	1.08	0.87	235	0.53	144	0.07	18	0.11	30	0.15	40	2.2	0.61
"	78	27.2	24.6	6.7	4.0	1.09	0.85	231	0.40	109	0.10	26	0.11	29	0.11	30	2.4	0.67
329	59	27.4	24.6	6.7	4.1	1.12	0.86	236	0.37	102	0.11	30	0.07	20	0.15	41	2.5	0.69
"	70	27.2	24.8	6.7	3.9	1.06	0.82	223	0.38	102	0.07	20	0.12	32	0.13	35	2.4	0.65
"	78	27.5	25.4	7.0	3.8	1.05	0.86	237	0.42	116	0.10	27	0.11	29	0.19	51	2.1	0.59
473	59	27.3	24.6	6.7	4.4	1.20	0.94	257	0.34	92	0.03	7	0.11	31	0.16	42	2.8	0.77
"	70	30.3	24.3	7.4	4.3	1.30	0.94	285	0.46	139	0.17	52	0.10	29	0.03	10	2.6	0.79
"	78	26.4	24.6	6.5	4.0	1.06	0.79	209	0.42	110	0.05	13	0.13	34	0.14	36	2.5	0.66
641	59	27.2	24.0	6.5	4.1	1.11	0.81	220	0.50	135	0.08	21	0.10	27	0.22	58	2.4	0.64
"	70	28.3	26.1	7.6	4.0	1.13	0.87	246	0.41	115	0.04	11	0.12	33	0.14	39	2.4	0.69
"	78	28.2	25.2	7.4	3.7	1.04	0.87	246	0.33	93	0.02	5	0.10	29	0.15	42	2.2	0.63
BC*	.....	23.6	25.2	5.9	3.0	0.71	0.43	102	0.37	88	0.05	11	0.10	25	0.19	45	1.9	0.44

* Barn-cured samples.

TABLE II

CONCENTRATION OF CERTAIN CONSTITUENTS OF BURLEY TOBACCO LEAF WEB AT DIFFERENT TIMES DURING THE AIR-CURING PROCESS, 1940 CROP, STATED AS PERCENTAGE OF THE DRY WEIGHT AND AS WEIGHT PER SQUARE METER OF LEAF AREA

Time	Relative humidity	Dry matter	Sulphated ash		Total nitrogen		Nicotine nitrogen		Asparagine nitrogen		Glutamine nitrogen		Ammonia nitrogen		Amino acid N (by diff.)		Residual nitrogen	
hr.	%	*gm.*	%	*gm.*	%	*gm.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*gm.*
0	.....	40.1	21.0	8.4	5.3	2.11	0.72	280	0.05	18	0.03	12	0.02	8	0.18	71	4.5	1.72
32	50	31.7	24.6	7.8	5.8	1.84	0.64	203	0.19	62	0.04	12	0.04	12	0.30	96	4.6	1.46
"	69	36.6	23.1	8.5	5.7	2.07	0.71	262	0.17	62	0.04	16	0.02	9	0.27	100	4.5	1.66
"	86	31.7	24.3	7.7	5.6	1.77	0.71	225	0.20	63	0.06	18	0.03	10	0.31	99	4.4	1.36
68	50	35.0	21.8	7.6	5.0	1.76	0.73	255	0.40	139	0.10	36	0.03	12	0.35	122	3.5	1.20
"	69	34.2	22.8	7.8	5.6	1.92	0.71	241	0.38	131	0.10	36	0.04	14	0.35	121	4.1	1.38
"	86	33.9	22.0	7.5	4.8	1.64	0.65	221	0.33	112	0.11	38	0.03	10	0.36	121	3.4	1.14
102	50	33.0	24.2	8.0	4.9	1.62	0.70	232	0.51	168	0.19	64	0.03	11	0.21	69	3.3	1.08
"	69	30.4	22.9	7.0	5.6	1.70	0.79	241	0.53	161	0.17	52	0.04	13	0.33	100	3.8	1.12
"	86	34.2	24.9	8.5	5.2	1.79	0.77	264	0.44	150	0.16	56	0.05	17	0.29	98	3.2	1.21
169	50	25.7	28.0	7.2	5.2	1.35	0.73	187	0.52	133	0.15	40	0.09	22	0.14	36	3.6	0.77
"	69	34.1	26.4	9.0	5.1	1.74	0.89	305	0.59	200	0.17	58	0.06	19	0.19	64	3.3	1.09
"	86	29.2	25.6	7.5	5.5	1.59	0.76	221	0.53	155	0.15	44	0.06	17	0.31	91	3.7	0.96
236	50	30.0	24.8	7.4	4.2	1.26	0.84	252	0.56	168	0.13	36	0.10	30	0.20	59	2.5	0.71
"	69	28.8	23.0	6.6	4.2	1.21	0.77	222	0.70	200	0.19	54	0.08	23	0.20	59	2.4	0.65
"	86	29.9	26.2	7.8	4.4	1.33	0.84	250	0.44	133	0.11	34	0.10	30	0.17	52	2.8	0.83
504	50	25.0	25.2	6.3	4.9	1.23	0.78	196	0.50	124	0.21	54	0.14	34	0.14	34	3.2	0.79
"	69	28.6	27.0	7.7	5.0	1.42	0.77	221	0.53	150	0.08	22	0.24	69	0.18	51	3.3	0.91
576	86	24.2	31.7	7.7	4.1	0.99	0.40	96	0.08	19	0.06	14	0.05	13	0.02	4	3.5	0.74
BC–1*	.....	31.6	26.5	8.4	4.5	1.43	0.90	284	0.42	131	0.11	34	0.14	46	0.12	39	2.9	0.90
BC–2*	.....	30.5	27.0	8.2	4.5	1.36	0.89	271	0.42	127	0.07	21	0.12	37	0.10	30	2.1	0.87

* Barn-cured samples.

little as possible. The midrib was removed from the leaves of the sample and the web was dried as rapidly as possible in a current of air at 67° C. The dried leaves were weighed, ground, and stored in waxed paper friction-top cartons. Moisture determinations were made and the dry matter per square meter of fresh leaf was calculated. LINK (5) found that in plants dried at 65° C. the only significant alteration of the nitrogenous constituents was a decrease in the amount of soluble nitrogen due to coagulation. None of the substances determined in this study are coagulated by heat. LINK did not determine ammonia or amide nitrogen in his study of the effect of drying.

Harvesting and sampling of the 1940 crop followed the same plan; only the points of difference will be given. On September 3, 1940 the plants were split and cut. The relative humidity of the three chambers was maintained at 50, 69, and 86 per cent. In 1939, no significant differences were observed in the composition of the samples cured at the different humidities; for this reason a wider range of relative humidities was selected for curing the samples from the 1940 crop. The results are given in table II. The analyses of the 1939 samples indicated that the chemical changes occurred more rapidly in the early stages of curing. Therefore most of the samples of the 1940 crop were taken during the early stages of curing. At cutting time two additional sticks were cut after their leaves had been measured as above and were placed in the barn to constitute the 1940 barn-cured samples.

ANALYSES

DRY MATTER.—The dry weight per square meter of leaf area was calculated from the leaf area of each sample, and the oven-dry weight of the leaf web of the sample.

SULPHATED ASH.—One gram of air-dry ground tobacco was weighed into an ignited and weighed porcelain crucible. Ten drops of a 5 to 3 solution of sulphuric acid in water were dropped on the sample. The sulphuric acid was added to prevent loss of potash by volatilization. The crucibles were placed in a cold muffle which was slowly heated to 700° C. Moisture was determined on the samples; and the grams of ash per 100 grams of oven-dry material (percentage) and the grams of ash per square meter of fresh leaf were calculated. A third determination was made if the duplicates did not agree within 3.5 per cent.

TOTAL NITROGEN.—A semi-micro Kjeldahl method was used. The digestion was made by a modification of the official GUNNING method of the Association of Official Agricultural Chemists (7). Fifty milligrams of air-dry, ground tobacco was introduced into a 100-ml. Kjeldahl flask. Three ml. of concentrated sulphuric acid containing 0.1 gm. of salicylic acid were added and the material was allowed to stand for 30 minutes with occasional shaking. One-half gram of sodium thiosulphate was added to effect the reduction of the nitrate nitrogen, and the material was warmed on an electric hot plate for 5 minutes. After cooling, 1 gm. of potassium sulphate and a small

piece of selenium were added and the mixture was digested 2 hours. On cooling, 10 ml. of water were added and the digested sample was washed into the KIRK apparatus (**4**), followed by 6 ml. of 40 per cent. sodium hydroxide. The ammonia liberated was steam distilled into a receiver charged with 5 ml. of 0.1 N sulphuric acid. The excess acid was back titrated with 0.05 N sodium hydroxide. After moisture determinations were made on the air-dry samples by drying duplicate 2-gm. samples for 16 hours in a vacuum oven at 70° C., the nitrogen was calculated as percentage of oven-dry material and as grams of nitrogen per square meter of fresh leaf area. Results were not considered acceptable unless the duplicates agreed within 3 per cent. It was necessary to have the samples finely ground in order to obtain this agreement.

NICOTINE NITROGEN.—Nicotine was determined by the AVENS and PEARCE (**1**) modification of the silicotungstic acid method. It was necessary to distill about 45 minutes instead of 30 minutes as recommended by AVENS and PEARCE in order to get duplicates agreeing within 2 per cent.

AMIDE NITROGEN.—Glutamine N and asparagine N were determined by the methods of VICKERY, PUCHER, and others (**12**). The amide N was determined on a hot-water extract of the dry tobacco prepared by heating at 80° C. for 10 minutes (**14**). In the case of both amides a third determination was made if the duplicates failed to agree within 6 per cent.

AMMONIA NITROGEN.—Ammonia N was determined by the method of PUCHER and co-workers (**9**). Nicotine interfered with the determination by forming a finely divided crystalline precipitate on reaction with the Nessler's reagent. This interference was overcome by precipitating the nicotine as nicotine silicotungstate following the distillation. The precipitate was removed prior to Nesslerization by centrifuging and an aliquot was taken for the determination. The precipitate could not be removed by filtering as the filter paper contained large enough quantities of ammonia to interfere seriously with the determinations. The excess silicotungstic acid caused no interference.

The nitrogen present as ammonia was calculated as percentage of oven-dry weight and as milligrams of ammonia N per square meter of leaf area. The determination was repeated if the duplicates failed to agree within 5 per cent.

AMINO ACID NITROGEN.—Amino acid nitrogen was determined by the method of PETERS and VAN SLYKE (**8**) on the hot-water extract of the dry tissue. Ammonia was removed by low-pressure distillation at 40° C. with light magnesium oxide prior to the determination of amino nitrogen in the VAN SLYKE manometric apparatus. A third determination was made when duplicates failed to agree within 1 per cent.

VICKERY and co-workers (**12**) found that 80 per cent. of the amide nitrogen of glutamine reacted with nitrous acid in the VAN SLYKE manometric apparatus. The total amino nitrogen was calculated by substracting 80 per cent. of the amide nitrogen of glutamine from the amino nitrogen found

by the VAN SLYKE method. The amino nitrogen other than that of the amides which will be designated hereafter as amino acid nitrogen was found by subtracting the amino nitrogen of the amides from the total amino nitrogen. Amino acid nitrogen was reported as the percentage of oven-dry weight and as milligrams of nitrogen per square meter of fresh leaf.

Results

Tables I and II give the results for the 1939 and 1940 crops, respectively. Some of these results are also presented in graphical form in figures 1 and 2, expressed as weight per square meter of fresh leaf. It should be noted in connection with these tables and figures that the central leaves of the plant, which were analyzed in this work, turned yellow in about 3 to 6 days, and brown in 7 to 12 days. The low relative humidity samples preceded the higher relative humidity samples in both color changes.

ANALYSES

DRY MATTER.—A marked decrease in dry matter occurred during the first seven days of curing, after which no significant change was observed. It seems evident that the regular initial sample for the 1939 crop is out of line and that the preliminary fresh leaf sample (designated as PFL) is more nearly correct; consequently, the lines in the figures are connected to this point though both values are recorded. If this value is used, it may be seen that the loss of original dry matter is about 30 per cent. in each year in which the experiment was conducted. The preliminary fresh leaf, the initial 1939, and the initial 1940 samples contained 43, 22, and 26 leaves, respectively; thus the initial 1939 sample would be expected to be the least accurate.

VICKERY *et al.* (**10, 13**) have reported decreases in dry matter during the curing of the primed leaves of a Connecticut shade-grown variety, amounting to 18 and 20 per cent. GARNER (**2**) observed decreases in dry matter of 12 and 15 per cent. during the curing of primed leaves and decreases of 25 to 30 per cent. during the curing of leaves on the stalk.

There are two reasons why stalk-cured leaves might be expected to lose more dry weight than primed leaves. One is that the stalk-cured leaves do not dry as rapidly as the primed leaves, therefore allowing greater opportunity for catabolic processes to go on which would give rise to volatile compounds. The other is that there is a chance for translocation to take place from the leaves to the stalk in tobacco cured on the stalk.

It has been found by other workers that, in plants living under adverse conditions, the growing points and meristematic tissue receive soluble nutrients at the expense of older and more mature tissue. For this reason, translocation might be expected to take place from the leaves to the stalks and thence to the living axillary buds or suckers in tobacco being cured with the leaves still attached to the stalk. It is possible to remove and plant "suckers" which have been on plants in the curing barn for as much as two months and to obtain good growth.

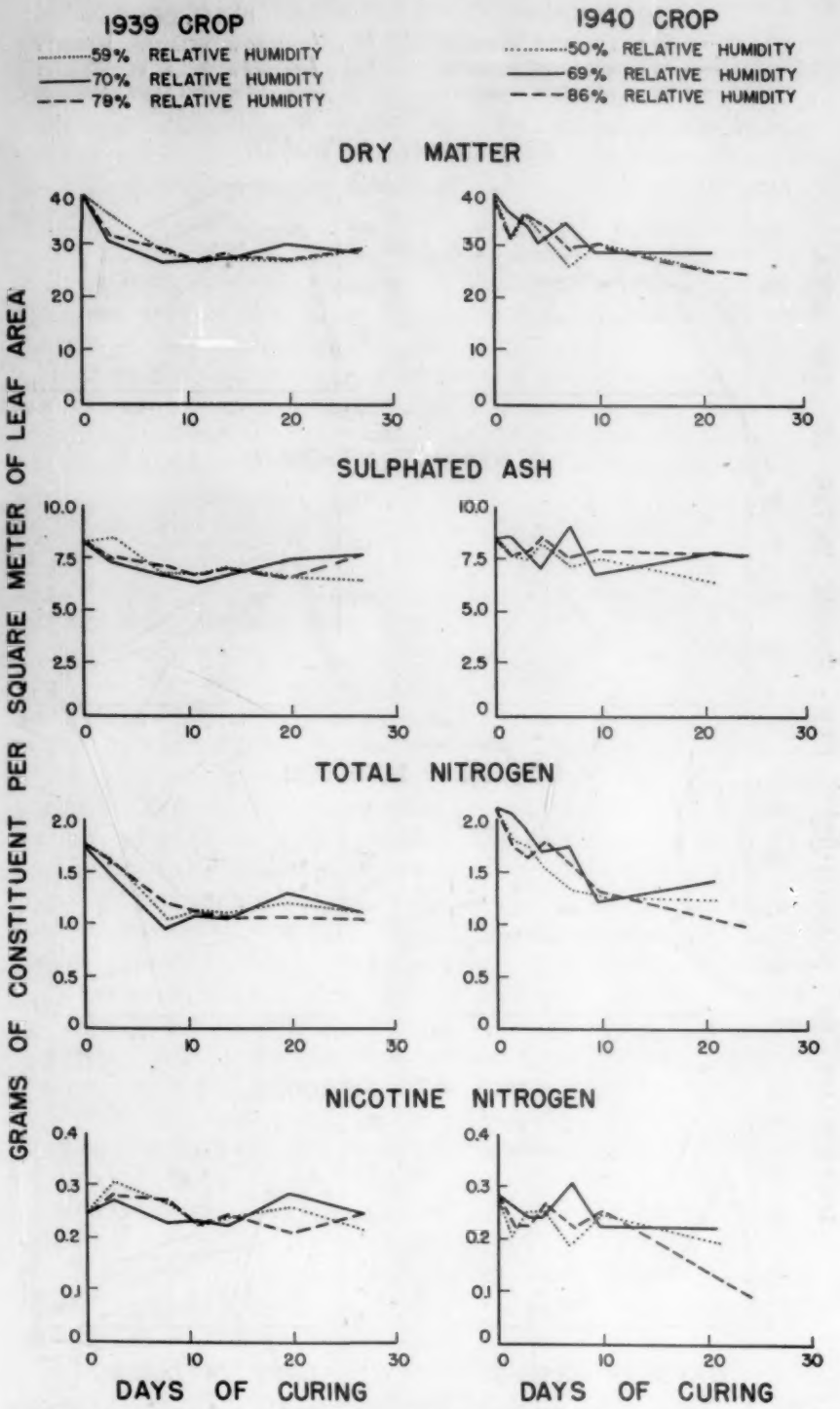


FIG. 1. Changes in the dry matter, sulphated ash, total nitrogen, and nicotine nitrogen content of Burley tobacco leaf during the air curing process.

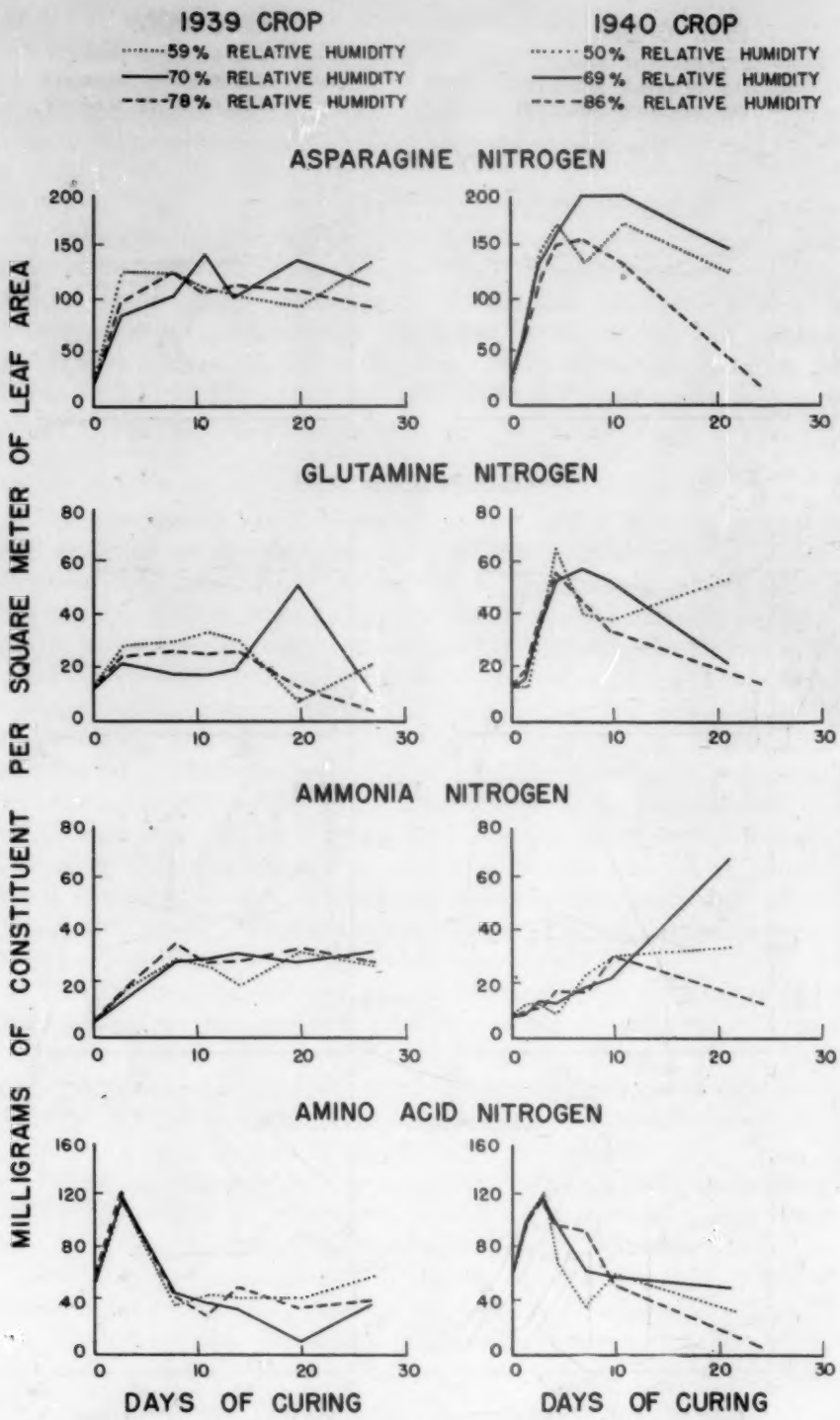


FIG. 2. Changes in the asparagine, glutamine, ammonia, and amino acid nitrogen content of Burley tobacco leaf during the air curing process.

SULPHATED ASH.—The results obtained by this determination are variable. If the initial samples are averaged and the samples from the later stages of curing are averaged, it may be concluded that the loss in ash content of the leaf web was about 12 per cent. GARNER (**2**), however, with fewer samples to indicate the variability of his data, attached significance to a decrease of about 9 or 10 per cent. of the original ash content of the whole leaf. He found decreases of 3.4 and 4.7 per cent. in the ash content of the leaf web.

TOTAL NITROGEN.—If the preliminary fresh leaf sample is considered to be the most accurate initial 1939 sample, as above, the loss in total nitrogen would appear to be about 38 per cent. in about 8 days in 1939 and about 41 per cent. in about 10 days in 1940.

GARNER (**2**) observed a larger decrease in total nitrogen in stalk-cured leaves than in primed leaves but he obtained evidence of loss of total nitrogen even in primed leaves; he attributed this to volatilization of ammonia. The additional decrease in total nitrogen in the case of stalk-cured leaves was attributed to translocation.

VICKERY (**10**) reported a decrease in total nitrogen in primed leaves of 15 per cent. This loss occurred before the leaves had all become brown. In a later investigation (**13**), his data did not indicate any decrease in total nitrogen during the same stages of curing.

NICOTINE NITROGEN.—The nicotine content of the samples expressed on a leaf area basis shows a slight downward trend as curing progresses. This change is probably too small to be significant except in the final 86 per cent. relative humidity sample; this had undergone "houseburn," which includes attack by microorganisms as well as further autolytic changes by the plant enzymes. This indicates that nicotine probably did not enter actively into the metabolism of the curing process. The nicotine values here reported are higher than similar values appearing in the literature for Burley tobacco. No satisfactory explanation can be offered for this difference. The method used was checked by means of samples previously run by another laboratory that used the A.O.A.C. method, and satisfactory agreement was obtained.

ASPARAGINE NITROGEN.—In 1939 the asparagine content of the curing tobacco increased to almost six times its initial value in the first three days of curing. After this no significant change was observed. No significant difference was found between the results at the different relative humidities. In 1940 the asparagine nitrogen increased to over eight times its initial value in about four days and then remained constant except for the 86 per cent. relative humidity sample. These statements do not imply that there was no further production of asparagine after the third or fourth day, but that the rate of destruction equaled the rate of production if it did continue. In the 86 per cent. relative humidity sample, the destruction of asparagine apparently continued after the production had ceased, resulting in a value about as low as the initial value.

GLUTAMINE NITROGEN.—The method for the determination of glutamine is rather inaccurate as only small amounts are present and it is calculated

by difference between two determinations neither of which was consistently duplicated within much less than 6 per cent. In 1939, the amount of glutamine nitrogen approximately doubled in the first three days of curing and then held approximately constant until about two weeks from the start of curing, after which it gradually decreased to about the initial value. The result obtained for the sample cured at 70 per cent. relative humidity for 473 hours (19.7 days) cannot be explained except as an experimental error.

In 1940, the glutamine, like the asparagine and to a lesser degree some of the other nitrogenous constituents, reached higher values than in 1939. The glutamine accumulated to over four times the original value and twice the maximum of 1939 in about four days and then gradually fell to about the same value as in 1939 except in the very low humidity sample. If the high final value on this sample is significant, it may be because this sample became very dry while the high glutamine content still existed, inhibiting further chemical change.[2]

The values found for amide content of leaf web were much more variable during the later stages of curing than in the early stages; it does not seem likely that analytical errors are altogether responsible.

VICKERY (**10**) found that the total amide nitrogen of the hot-water extract of whole tobacco leaves increased from 0.04 gm. per 1000 gm. of fresh leaf to about 0.4 gm. during curing. Later (**13**), he found that the total amide nitrogen increased from 0.2 gm. per 1000 gm. of fresh leaf to about 1.0 gm. during the same stages of curing.

There are two views concerning the formation of amides in plants. The view of VICKERY (**11**) is that amide metabolism is a phase of the general respiratory activity of the tissues. The older view of PRIANISCHNIKOW was that the function of amides was to provide a means of disposal of ammonia which otherwise might accumulate in high concentrations and prove toxic. The nitrogen content of the leaves was greater in the 1940 than in the 1939 crop. Also a greater decrease in total nitrogen occurred in the samples from the 1940 crop. This means that probably more catabolic changes occurred which might give rise to ammonia, a form of nitrogen thought to be essential in amide synthesis. A much larger quantity of amides was found in the 1940 samples but this could be due to decomposition in the 1939 samples as previously indicated. If the amount of amides formed in 1940

[2] Since this work was completed, glutamine has been re-determined on some of the 1940 cured samples. The values did not agree with those previously found; they were so low that they stimulated speculation as to the stability of the amides in air-dry plant tissues when stored for considerable lengths of time. Ten months elapsed from the time the samples were taken from the 1939 crop until these samples were analyzed for amides. Only 4 months elapsed from the time the 1940 samples were taken until they were analyzed for amides. The values for amides of the 1939 crop were much lower than for the 1940 crop and, in view of the values obtained for the 1940 crop nine months after sampling, one is led to the conclusion that the amides may decompose during storage under such conditions. This casts doubt upon the results of this experiment and possibly upon VICKERY'S results (**14**).

was larger because of the increased protein decomposition it still does not prove conclusively whether or not detoxification was actually responsible.

The increase in amide nitrogen appears to have been more immediate than the increase in ammonia nitrogen, particularly in the results from the 1940 crop, where the early samples were taken at more frequent intervals and the analyses are generally more reliable. It may be that the amide nitrogen ceased to rise after about the fifth day because of failure of the supply of α keto acids.

AMMONIA NITROGEN.—During curing of the 1939 crop, the ammonia nitrogen increased to about six times its original value, in the first week, and then remained about constant in all samples. In 1940 the same level was reached after ten days. A high value was obtained for the final 1940 sample at 60 per cent. relative humidity, but this is probably an error. The sample cured at 86 per cent. relative humidity lost ammonia in the later stages of curing, similar to its loss of other soluble nitrogenous constituents.

AMINO ACID NITROGEN.—During curing of the 1939 and 1940 crops, the amino nitrogen other than that contributed by the amides reached a maximum in about three days, after which it decreased, reaching values as low or lower than the initial in another three days in 1939 and somewhat more slowly in 1940. There is probably no other significant change except in the final 1940 high-humidity sample, which is low in amino acid nitrogen as well as other soluble nitrogen components. The hydrolysis of part of the protein to amino acids is one of the first changes that take place in the nitrogen compounds of the curing leaf; this might be expected. It is followed by a rapid conversion of the amino acids to other forms.

During curing of the 1940 crop a decrease of amino nitrogen (other than that of amides) was observed between the third and tenth days, amounting to about 60 mg. per square meter. An increase of two-thirds this amount was observed in the nitrogen of amide groups of asparagine and glutamine. The remainder of the decrease in total amino nitrogen can be accounted for only by translocation or volatilization. During curing of the 1939 crop, a decrease in amino acid nitrogen of about 75 mg. occurred. As practically no net synthesis of amides was detected during this period, the loss probably occurred by volatilization or translocation or both.

RESIDUAL NITROGEN.—If the sum of the asparagine N, glutamine N, ammonia N, amino acid N, and nicotine nitrogen is deducted from the total N, a figure is obtained which is here designated as the residual N. This residual N is probably chiefly protein N and nitrate N. It decreased by about 32 per cent. in the 1939 crop and by about 60 per cent. in the 1940 crop, which had a higher initial nitrogen content. The final value reached is not far different in the two years. In this, as in most other cases, the loss all occurred in the first ten days.

BARN-CURED SAMPLES.—The barn-cured samples were analyzed for the same components as were the samples cured in the curing chambers. The

findings are given in the tables along with the other data, designated BC. The values found agreed, for the most part, with those for the samples cured in the chambers.

Discussion

If we attempt to draw general conclusions from the data presented in the graphs, it will be seen that no significant differences are evident between the tobacco cured at different relative humidities with the single exception of the final sample cured at 86 per cent. This sample was so badly injured that even a person unfamiliar with tobacco would pick it out immediately. The other samples were sufficiently different to be distinguishable by any good tobacco grader. Since the data do not show any such difference, it is obvious that the analyses did not include those substances which are responsible for quality as determined by commercial grade. On the other hand, they did include substances which changed markedly during the curing process. It would appear, as one might expect, that the first change was a hydrolysis of the protein to amino acids which, in turn, were either translocated or yielded ammonia and amides. The increase in ammonia concentration was rather slow at first, but it would appear that ammonia was being produced, as it was probably an intermediate in the formation of the amides. It is also the most volatile nitrogen compound whose presence would be expected. In the 86 per cent. relative humidity sample the loss of all soluble nitrogen compounds was the most complete. Fungus growth was visible on this sample. Probably various microorganisms combined with the plant enzymes to produce the more complete autolysis in this sample.

It is evident that the loss of ash occurred by translocation. Although it cannot be assumed that the movements of inorganic salts and soluble nitrogen compounds would necessarily be the same, the total nitrogen content of the leaves fell by about 40 per cent. while the ash content was falling about 12 per cent.; consequently, it seems possible that some of the nitrogen was lost by volatilization.

After the amide concentration had ceased to rise at about the fourth day, the ammonia continued to rise until about the eighth or tenth day. About this time all significant changes ceased except in the sample which underwent "house-burn." This sample lost additional nitrogen in all soluble forms which were determined, including nicotine. By the end of the curing period, this tobacco was dark colored, had the characteristic odor of "house-burn" known to all tobacco men, and had fungus colonies on it. It is possible that microorganisms may have used the soluble nitrogen compounds in the synthesis of insoluble compounds or undetermined soluble compounds. There was no significant change in the undetermined forms reported in the tables as residual nitrogen. This indicates either that no further attack was made by the plant enzymes or the microorganisms on the leaf protein, or that the amount of protein formed by the microorganisms from soluble sources was about equal to the amount broken down.

Summary and conclusions

This study was made to determine some of the chemical changes that occur in leaves of White Burley tobacco during curing on the stalk, under automatically controlled conditions of temperature and relative humidity. Samples taken at different stages of curing were analyzed for ash, total nitrogen, nicotine nitrogen, asparagine nitrogen, glutamine nitrogen, ammonia nitrogen and amino acid nitrogen. The results are expressed as weight per square meter of fresh leaf. The following conclusions are justified:

1. The dry matter content of the leaf web decreased by about 30 per cent. during the early stages of curing.

2. The total nitrogen decreased by about 40 per cent. of the original amount in the early stages of curing.

3. A rapid increase occurred during the early stages of curing in all forms of soluble nitrogen determined except nicotine.

4. The asparagine, glutamine, and ammonia nitrogen remained high except in leaves cured at very high relative humidity (86 per cent.).

5. The amino acid nitrogen determined by subtracting the amino nitrogen of the amides from the total amino nitrogen decreased from its maximum to below its original concentration. Some of this amino acid nitrogen was used in amide synthesis, some was translocated and some may have been volatilized.

6. The nicotine content remained practically constant throughout the cure except in the "houseburned" sample, indicating that nicotine does not enter actively into the normal metabolism of the curing process.

7. The leaves cured at 86 per cent. relative humidity lost most of their soluble nitrogen during the later stages of curing. This loss is probably due in part to the action of the enzymes of the plant and in part to the action of microorganisms growing on the leaves.

8. Except for the sample cured at 86 per cent. relative humidity, there is no evidence of change in composition after the leaf has burned brown. This indicates that changes due to plant enzymes and microorganisms are both nearly stopped as soon as the tissue has become dry.

KENTUCKY AGRICULTURAL EXPERIMENTAL STATION
LEXINGTON, KENTUCKY

LITERATURE CITED

1. AVENS, A. W., and PEARCE, G. W. Silicotungstic acid determination of nicotine. Ind. & Eng. Chem. Anal. Ed. 11: 505–508. 1939.
2. GARNER, W. W., BACON, C. W., and FOUBERT, C. L. Research studies on curing of leaf tobacco. U.S.D.A. Bull. 79. 1914.
3. JEFFREY, R. N. The effect of temperature and relative humidity during and after curing, upon the quality of White Burley tobacco. Kentucky Agr. Exp. Sta. Bull. 407. 1940.

4. KIRK, P. L. A one piece glass Micro-Kjeldahl distillation apparatus. Ind. & Eng. Chem. Anal. Ed. **8**: 223–224. 1936.
5. LINK, K. P., and SCHULZ, E. R. Effects of the method of desiccation on the nitrogenous constituents of plants. Jour. Amer. Chem. Soc. **46**: 2044–2050. 1924.
6. O'BANNON, L. S. A laboratory for use in determining the influence of various temperatures and relative humidities on the final quality of air-cured tobacco. Heating, Piping, and Air Conditioning **11**: 699–702. 1939.
7. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed. Washington. 1940.
8. PETERS, J. P., and VAN SLYKE, D. D. Quantitative clinical chemistry. Vol. II. Methods. Williams and Wilkins Company, Baltimore, 1932.
9. PUCHER, G. W., VICKERY, H. B., and LEAVENWORTH, C. S. Determination of ammonia and of amide nitrogen in plant tissue. Ind. & Eng. Chem. Anal. Ed. **7**: 152–156. 1935.
10. VICKERY, H. B., and PUCHER, G. W. Chemical investigations of the tobacco plant. II. The chemical changes that occur during the curing of Connecticut shade-grown tobacco. Connecticut Agr. Exp. Sta. Bull. 324. 1931.
11. ————, and ————. The metabolism of amides in green plants. II. The mechanism of amide synthesis. Jour. Biol. Chem. **128**: 703–713. 1939.
12. ————, ————, CLARK, H. E., CHIBNALL, A. C., and WESTALL, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. **29**: 2710–2720. 1935.
13. ————, ————, WAKEMAN, A. J., and LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. Carnegie Institute of Washington. Pub. no. 445, Parts I and II. 1933.
14. ————, ————, ————, and ————. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and in darkness. Connecticut Agr. Exp. Sta. Bull. 399. 1937.
15. WOLF, F. A., and GROSS, P. M. Flue-cured tobacco; a comparative study of structural responses induced by topping and suckering. Bull. Torrey Bot. Club **64**: 117–131. 1937.

A QUANTITATIVE STUDY OF CHLOROSIS IN CHLORELLA UNDER CONDITIONS OF SULPHUR DEFICIENCY

GABRIEL R. MANDELS

(WITH TEN FIGURES)

Introduction

The rôle of chlorophyll in the process of photosynthesis is not clearly understood.[1] Present evidence indicates that under certain conditions there is a direct relation between chlorophyll content and photosynthesis in Chlorella cells (**1, 4**). On the basis of experiments with intermittent light, EMERSON and ARNOLD (**2**) suggested the existence of a chlorophyll unit in the light reaction in photosynthesis. Later work has shown, however, that such a relation does not always exist and that the concept of a fixed chlorophyll unit in photosynthesis is no longer tenable (**3**). It would be of considerable interest to know how the efficiency of chlorophyll changes with varying degree of chlorosis. A thorough investigation of this type is not available. Prerequisite to such a study is a quantitative analysis of the development of chlorosis and of recovery from chlorosis. The present work is an investigation of this nature under conditions of sulphur deficiency.

Methods

The strain of Chlorella used in this investigation was originally isolated from soil by WANN (**12**) and is known as Chlorella no. 11. It is the same strain that was used in the studies of HOPKINS and WANN (**5, 6, 7**), FLEISCHER (**4**), PEARSALL and LOOSE (**10**), LUDWIG (**9**), and KENNEDY (**8**). FLEISCHER'S (**4**) modification of the nutrient solution employed by EMERSON was used, the composition being: Na citrate—1.00 gm.; KNO_3—1.25 gm.; $MgSO_4 \cdot 7H_2O$—2.46 gm.; KH_2PO_4—1.22 gm.; Fe (as $FeCl_3$)—0.01 gm.; glucose—15 gm.; H_2O—1 l. With the exception of the Na citrate (Baker and Adams, C. P.), (fig. 1) all the chemicals used were J. T. Baker's C.P. analyzed. The sulphur deficient medium was prepared by replacing the $MgSO_4$ with an equivalent amount of $MgCl_2$. Three-liter Florence flasks containing 1.5 l. or 12-l. flasks containing 8 l. of nutrient solution were used as culture vessels. The cultures were sterilized by autoclaving at 15 lb. for 25 minutes or for 35 minutes when the larger vessels were used. The initial pH of the cultures after sterilization was about 5.3. Suspensions of cells grown in a full nutrient solution were employed for inoculation. In some experiments a suspension of cells in sterile distilled water was used, the cells having been rinsed previously in distilled water (three times, by repeated centrifugation and decantation, observing aseptic precautions).

Large culture vessels were used so that the studies could be confined to cultures in which the population density did not exceed 2,000 cells/mm.[3]

[1] Examination of the literature leads to the conclusion that there is little direct data concerning the rôle of chlorophyll.

The possibility of secondary effects due to significant changes in the composition of the nutrient solution was thus minimized.

The cultures were grown in an insulated light chamber at 25 or 30° C. ± 0.5°. A 500-watt tungsten filament bulb placed in a reflector over the top of the chamber served as a light source. Infra-red radiation was filtered out by a ½-inch layer of circulating water. The light intensity was of the order of 500 foot candles as measured on the floor of the chamber with a Weston Illuminometer. The cultures were shaken for 5 minutes every half hour, a mobile platform being in the chamber.

The population density of the cultures was determined with a haemocytometer. A photoelectric colorimeter was used to determine the chlorophyll content of methanol extracts of the cells. Interference by carotenoids was avoided by using a Corning signal red filter having a cut-off at 6300 Å. The colorimeter was calibrated with a preparation of purified cholorphyll extracted from corn leaves following the procedure of SCHERTZ (**11**). Chlorophyll determinations were always made immediately after removal of the sample from a culture.

EXPRESSION OF DATA

The population density of the culture (N) is in terms of cells per mm.^{3} of culture. The rate of cell division is the slope of the curve giving the logarithmic increase in cell number per hour, $\left(\frac{\Delta \log N}{\Delta T}\right)$. Chlorophyll is expressed in micromoles per 50 ml. of culture, (C), or on a basis of μ moles per 10^9 cells (C_n). The rate of chlorophyll formation is the slope of the curve giving the logarithmic increase in chlorophyll, $\frac{\Delta \log C}{\Delta T}$.

Results

DEVELOPMENT OF CHLOROSIS IN SULPHUR-DEFICIENT CULTURES

Practically no growth will occur if a suspension of washed cells, grown in a full nutrient solution, is used to inoculate a culture to which no sulphate has been added. Thus neither previous accumulation of sulphur in cells nor sulphur impurities in the chemicals used, are significant. In the experiments reported here, sufficient sulphate was added to support growth up to a population density of the order of 2,000 cells per mm.^{3}

Data showing typical behavior during growth in a sulphur deficient culture are presented in figure 1. It is seen that cell division and chlorophyll formation gradually cease. It is significant that the rate of chlorophyll formation decreases more rapidly than the rate of cell division. Thus there is an initial decrease in the amount of chlorophyll per cell while chlorophyll formation is still occurring. Even after cessation of chlorophyll formation there is a slight increase in cell number. This results in a further decrease in the chlorophyll content per cell. Development of chlorosis continues due to decomposition of chlorophyll in the cells. Decomposition is initiated about the time cell division ceases.

To get a quantitative measure of the degree of chlorosis the chlorophyll deficit of the chlorotic cells can be expressed as a percentage of the normal chlorophyll complement.

$$\text{Percentage of chlorosis} = \frac{C_{N_0} - C_{N_1}}{C_{N_0}} \times 100$$

where C_{N_0} = μ moles chlorophyll/10^9 normal cells = 3.50; C_{N_1} = μ moles chlorophyll/10^9 chlorotic cells. On this basis it is found that when chlorophyll synthesis stops, the cells are 45 per cent. chlorotic. The slight increase in cell number found after this point results in 64 per cent. chlorosis when cell division stops. These values for the degree of chlorosis at cessation of chlorophyll formation and at cessation of cell division appear to be quite

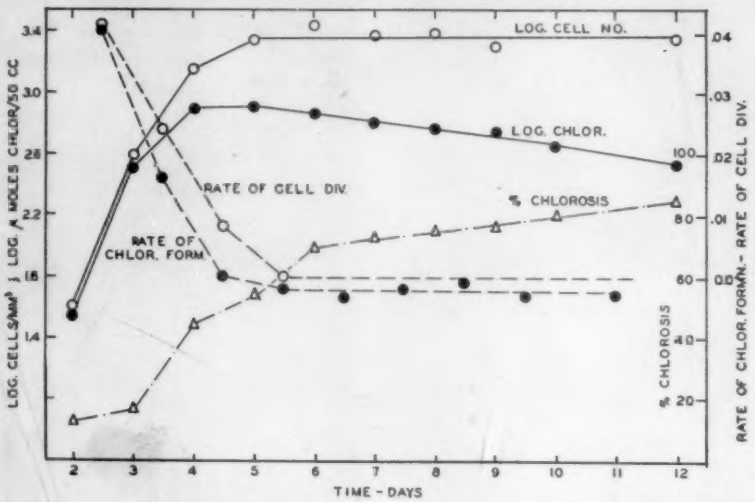

LOG. CELL NO.
LOG. CHLOR.
RATE OF CELL DIV.
% CHLOROSIS
RATE OF CHLOR. FORM.
LOG. CELLS/MM³ & LOG. μ MOLES CHLOR/50 CC
% CHLOROSIS
RATE OF CHLOR. FORMN. - RATE OF CELL DIV.
TIME - DAYS

FIG. 1. Growth in culture lacking sulphur showing the development of chlorosis at 25° C. (The ordinate log. chlorophyll has been shifted up 4.75 units.)

constant. Decomposition of chlorophyll results in a gradual increase in the degree of chlorosis. Complete loss of chlorophyll (100 per cent. chlorosis) is attended by death of the cells since they no longer respond to addition of sulphate. Such cells contain significant amounts of carotenoids. The carotenoids decompose gradually in the dead cells.

Microchemical tests of the chlorotic cells with Sudan IV in lactophenol and with I–KI show an accumulation of considerable amounts of fat and little or no starch. This behavior is also found if sugar is omitted from the medium. Normal cells in full nutrient solution do not contain sufficient fat or in a form to be demonstrated with Sudan IV.

RECOVERY FROM CHLOROSIS

To follow the process of recovery an aliquot of a sterile, standard solution of K_2SO_4 was added aseptically to cultures containing sulphur deficient, chlorotic cells. Samples were removed aseptically at intervals for chloro-

phyll determinations. In some experiments lasting only about 24 hours, aseptic precautions were not observed. In such cases bacterial or fungal growth was insufficient to have any noticeable effect. The cultures were kept in the growth chamber during recovery.

Chlorophyll formation is the first visual evidence of recovery—(macro- or microscopic). This can be detected about five hours after the addition

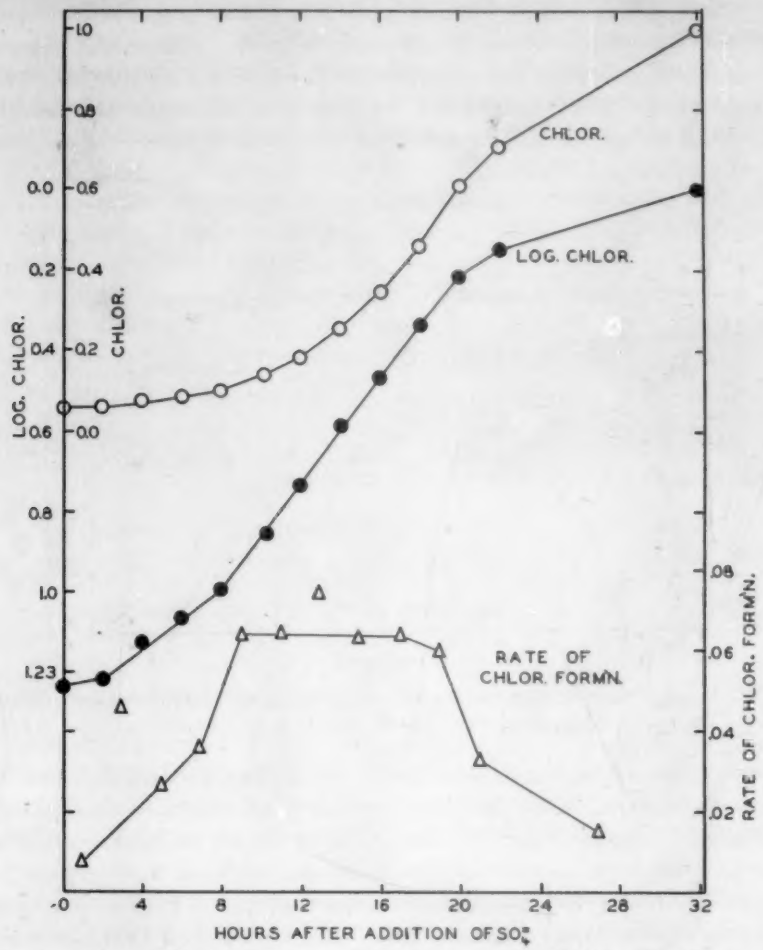


FIG. 2. Chlorophyll formation during recovery from sulphur deficiency. (2090 cells per mm.^{3}; 84 per cent. chlorosis; 1.67 p.p.m. of S as $SO_4^=$; 25° C.)

of sulphate. In a culture having a population of 1500 cells/mm.^{3}, formation of chlorophyll occurs at concentration of sulphate as low as 3×10^{-7} M (0.01 p.p.m. of S). Recovery does not proceed sufficiently to permit cell division unless about 0.1 p.p.m. of S is added. If cell division is to occur there is a lag of about 24 hours before autospore formation is noted. Growth by increase in cell volume, as noted by microscopic observation, occurs before the cells divide (no quantitative measurements were made).

Typical data showing the formation of chlorophyll are presented in figure 2. The process of chlorophyll formation during recovery can be separated into four phases:

1. A lag period of a few hours (usually about four hours) between addition of sulphate and chlorophyll formation.

2. A period of acceleration in chlorophyll synthesis during which the rate of formation increases from zero to a maximum which is attained at about the tenth hour.

3. A period of logarithmic increase in chlorophyll. It should be noted that the maximum rate of synthesis which is maintained during this period (about 10 hours) is considerably in excess of the rate of chlorophyll formation during normal growth in full nutrient solution.

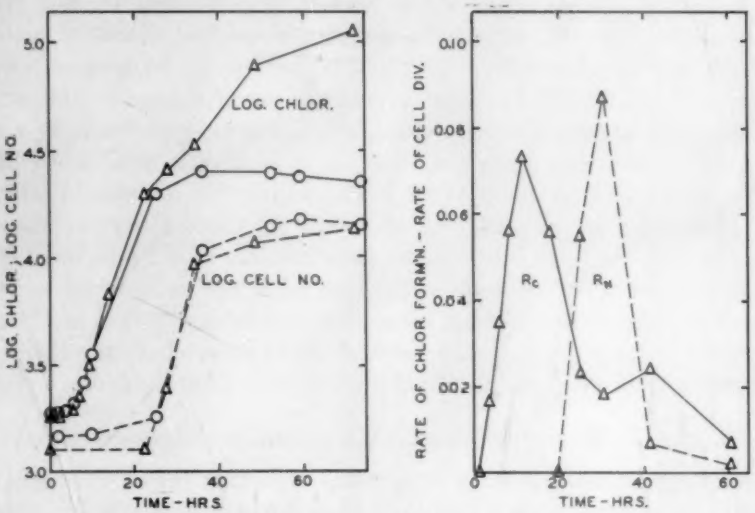


FIG. 3. Chlorophyll formation and cell division during recovery. (1.93 p.p.m. of S ○; 10.0 p.p.m. △; broken line — log. cell no.; solid line — log. chlorophyll) (70 per cent. initial chlorosis; 25° C.)

FIG. 4. Rate of chlorophyll formation (R_c) and rate of cell division (R_N) during recovery in 10 p.p.m. of S culture.

4. A period of decrease in rate of synthesis. In the culture under consideration here, sufficient sulphate for continued recovery was not present so that the rate decreases to zero.

Data showing recovery with respect to both chlorophyll formation and cell division in two comparable cultures at different concentrations of sulphate are given in figures 3 and 4. There is a rapid increase in chlorophyll in both cultures. The cells, initially about 70 per cent. chlorotic, contain several times the normal chlorophyll complement at the time cell division is initiated. At a concentration of 1.93 p.p.m. of S there was insufficient sulphate for continued recovery. Thus there is only a slight increase in chlorophyll after the inception of cell division. A rapid decrease in the amount

of chlorophyll per cell is found during the spurt in cell division (from 8.66 to 1.88 μ moles/10^9 cells). Subsequently there is a gradual decrease due first to cell division and finally to decomposition. Thus the cells have returned to a condition of sulphur deficiency.

At the concentration of 10 p.p.m. further recovery was possible, synthesis of chlorophyll continuing after the initial spurt in cell division. The sudden decrease in slope of the log chlorophyll curve at the time cell division starts should be noted (fig. 4).

It should be observed that while there is a relation between the amount of chlorophyll formed and the amount of sulphate added, there is none with respect to increase in cell number. The chlorophyll yield will be considered in more detail later.

Since a detailed analysis of the process of cell multiplication during recovery is not directly pertinent to the scope of this paper, a brief summary will be adequate. Although chlorophyll formation can be detected when as little as 0.01 p.p.m. of S is added to a suspension of chlorotic cells, at least ten times this amount must be added before recovery proceeds to a stage where cell division will occur (in a culture of 1500 cells/mm.^{3}). With this minimum amount of sulphate there will be a nine-fold increase in cell number. Increasing the sulphate concentration ten times above the minimum for cell division will still result in the same increase in cell number. If still greater amounts of sulphate are added then there will be an initial nine-fold increase in cell number followed by a short lag before further cell division continues. Thus at least at low concentrations of sulphate the increase in cell number is not proportional to the amount of sulphate added.

Factors influencing chlorophyll formation during recovery

Effect of initial degree of chlorosis.—To study the effect of length of exposure of cells to a condition of sulphur deficiency upon subsequent recovery, a culture containing 8 l. of nutrient solution was used. Samples were removed aseptically at intervals corresponding to 6.83, 8.88, and 12.21 days after inoculation. Chlorophyll formation was studied after adding 1.0 p.p.m. of S as $SO_4^=$. Thus recovery was followed using portions of the same suspension of cells having chlorophyll complements equivalent to 22, 28.5 and 45 per cent. chlorosis. Although the first sample of cells was only 22 per cent. chlorotic, cell division had ceased. This is contrary to the preceding data in which cell division ceased when the cells were about 65 per cent. chlorotic. This anomalous behavior was probably due to inadequate provision for gas exchange during the development of the culture. Presumably the low O_2 tension suppressed the amount of growth by cell division while chlorophyll formation did not suffer similar interference. The results reported below have been substantiated using "normal" sulphur deficient cells.

The data are summarized in figures 5 and 6. During the first few hours after addition of sulphate there is a decrease in chlorophyll. The extent of

this decomposition is greatest in the slightly chlorotic cells and decreases progressively in the two subsequent series. The interpretation of this phenomenon is not clear. The rate is too great to be normal decomposition.

The recovery curves (fig. 5) show that despite the relatively large differences in initial chlorophyll content the maxima reached are practically the same in all three series. Thus the number of moles of chlorophyll formed per mole of sulphate added is determined to a slight extent by the initial degree of chlorosis, the exact figures being 0.307, 0.356, and 0.360 for the three series.

Consideration of the rate of chlorophyll synthesis in the three series brings out another interesting relation (fig. 6). The curves show that the maximum rate of synthesis attained in each of the series is higher as the initial degree of chlorosis increases.

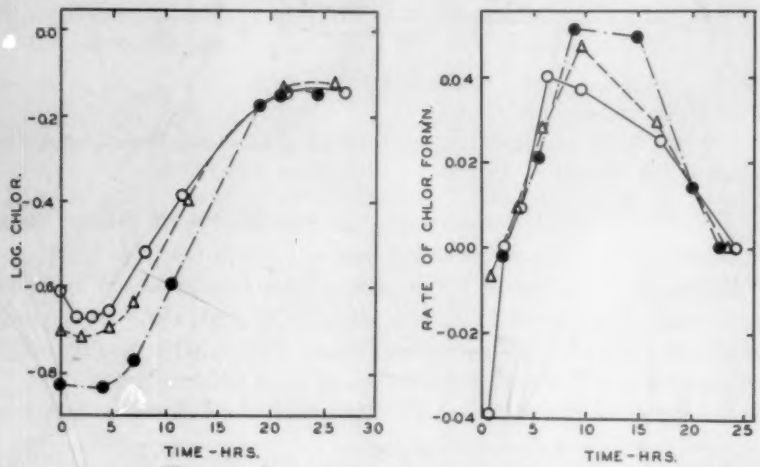


FIG. 5 and 6. Effect of initial degree of chlorosis on chlorophyll formation during recovery. (○ — 22 per cent.; △ — 28.5 per cent.; ● — 45 per cent. chlorosis.) (1.0 p.p.m. of S; 1520 cells 1 mm.^{3}; 25° C.)

EFFECT OF SULPHATE CONCENTRATION ON THE RATE OF CHLOROPHYLL SYNTHESIS.—Eight liters of a suspension of sulphur deficient cells were divided into equal portions to which different amounts of sulphate were added. The vessels were then placed in the growth chamber under a condition of darkness to minimize chlorophyll decomposition. Since identical suspensions of cells were used, sulphate concentration was the only variable. Data showing the changes in rates of synthesis in the cultures are summarized in figure 7. Two effects are to be observed. First, the effect on the acceleration of chlorophyll synthesis—increasing the concentration up to about 0.5 p.p.m. of S results in a more rapid increase in rate of synthesis. Greater increments have little or no effect (see also fig. 3). Secondly, it is seen that the maximum rate of synthesis attained increases as sulphate concentration is raised to about 0.5 p.p.m. of S. This relation is seen more exactly in figure 8. From 0.05 to 0.5 p.p.m. the relation $R_c = K \log [SO_4^=]$ obtains (where R_c = maxi-

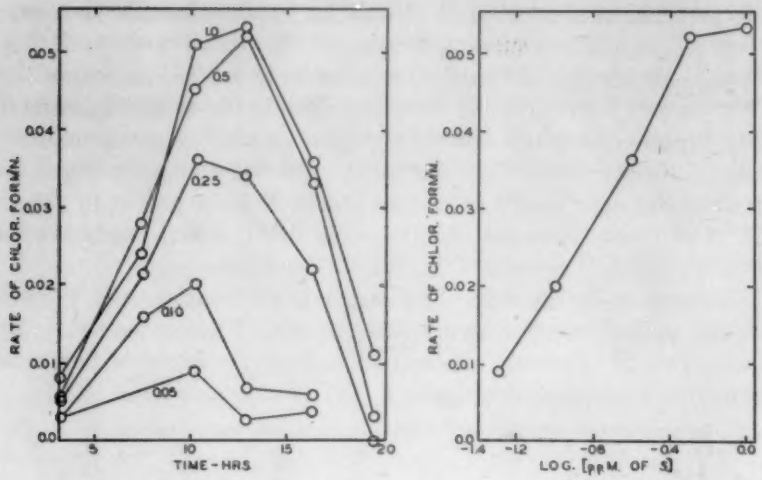


FIG. 7. Effect of sulphate concentration (figures in p.p.m. of S) on chlorophyll formation during recovery.

FIG. 8. Maximum rate of chlorophyll synthesis attained at different concentrations of sulphate during recovery.

mum rate of chlorophyll formation). At concentrations greater than 0.5 p.p.m. there is little or no effect on the maximum rate (see also fig. 3).

EFFECT OF LIGHT.—One of two similar cultures was darkened by enclosing it in several layers of black cloth with black rubberized cloth on the outside. Both cultures were then placed in the light chamber after adding sulphate. Any temperature differences were not considered as being significant. The results are shown in figures 9 and 10. Although the differences between the

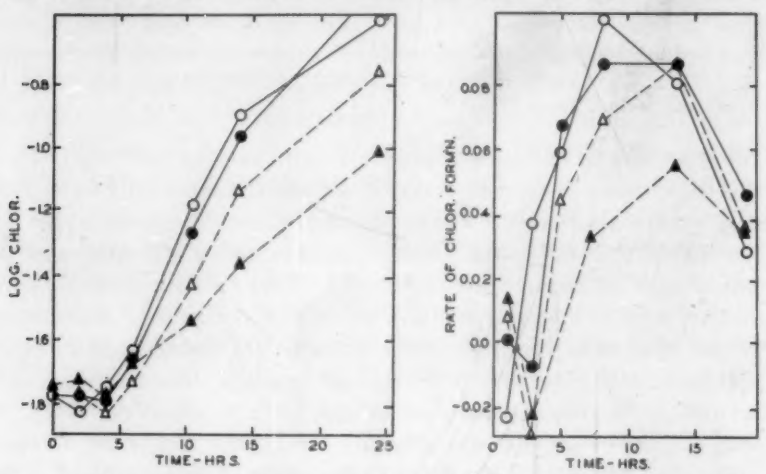


FIG. 9. Effect of light and source of sulphur on chlorophyll formation during recovery. (○ — $SO_4^=$ in light; ● — $SO_4^=$ in dark; △ — H_2S in light; ▲ — H_2S in dark.)

FIG. 10. Effect of light and source of sulphur on the rate of chlorophyll formation. (Symbols same as in fig. 9.)

two treatments are not very great, it should be noted that there is a slightly longer lag in the dark and also that the maximum rate of chlorophyll synthesis attained is slightly less in the dark. Another effect is brought out in table I which shows the chlorophyll yield to be slightly greater in the dark. Limited data show that during recovery the rate of cell division is retarded to a much greater extent in the absence of light than is synthesis of chlorophyll.

SOURCES OF SULPHUR AVAILABLE.—Some qualitative studies were made to test the availability of different sulphur compounds for recovery from

TABLE I

MOLES CHLOROPHYLL FORMED* DURING RECOVERY PER MOLE OF SULFATE ADDED

SULPHATE CONCENTRATION	MOLES $SO_4^=$ ADDED/10^9 CELLS	INITIAL CHLOROSIS	CELL DIVISION	MOLES CHLOR. FORMED/MOLE $SO_4^=$ ADDED
		%		
Light 1.93 p.p.m. of S	42.0	70.5	+	0.310
1.0	20.4	22.0	+	0.307
1.0	20.4	28.5	+	0.356
1.0	20.4	45.0	+	0.360
1.0	24.4	68.6	+	0.394
0.5	10.2	22.0	+	0.314
0.5	10.2	28.5	+	0.357
0.5	10.2	45.0	+	0.415
0.167	2.74	85.4	−	0.291
0.10	2.44	68.6	−	0.321
0.01	0.244	68.6	−	0.321
Dark 1.0	20.4	52.0	?	0.280
0.5	10.2	51.8	?	0.405
0.25	5.1	51.8	?	0.483
0.10	2.04	51.8	?	0.483
0.05	1.02	51.8	?	0.420
0.05	1.02	51.8	?	0.385
0.025	0.51	51.8	?	0.456

* Amount of chlorophyll formed = max. chlorophyll during recovery − initial chlorophyll.

Average yield in light—0.34 moles chlor./mole sulphate.
" " " dark—0.42 " " " " "

sulphur deficiency. All the organic compounds tested were unavailable (cysteine, glutathione, methionine, thioglycollic acid, thiamin, potassium thiocyanate). The possibility that toxic concentrations of these substances were used is unlikely since recovery occurred in control cultures to which both sulphate and the various compounds were added. All of the inorganic compounds tried were available for recovery (hydrogen sulphide, thiosulphate, pyrosulphate, sulphite, persulphate). Tests also showed that sulphate could not be replaced by tellurate or selenate.

While it would be very interesting to have quantitative data showing the length of the lag period, the rate of chlorophyll synthesis and the chlorophyll yield during recovery when different sulphur compounds were used, comparative studies were made only with H_2S and $SO_4^=$. The results of this study are summarized in figures 9 and 10. The curves show a longer lag

when sulphur is supplied as H_2S than as sulphate and also a slower rate of synthesis, these effects being more pronounced in the dark.

EFFECT OF TEMPERATURE.—No detailed study was made of the effect of temperature on chlorophyll formation during recovery. Data are available, however, to show that: (1) The maximum rate of synthesis attained at 30° C. is significantly higher than at 25° C. (2) Recovery does not occur (chlorophyll formation or cell division) at 10° C. or lower, although the cells are not permanently injured by this treatment.

NECESSITY OF OXYGEN.—Results of several experiments showed that no recovery occurs if the atmosphere in equilibrium with the culture medium was unpurified tank nitrogen (probably containing about 0.5 per cent. O_2).

CHLOROPHYLL YIELD DURING RECOVERY.—It is obvious that the extent of recovery must be some function of the amount of sulphate added and data presented already have anticipated the conclusion that the amount of chlorophyll formed shows some proportionality to the amount of sulphate added. Results from several different experiments showing the amount of chlorophyll formed during recovery per mole of sulphate added are summarized in table I. A consideration of the data obtained during recovery in the light shows that the chlorophyll yield is more or less constant over a wide range in concentration of sulphate—from 0.01 to 1.93 p.p.m. of S. About 0.34 moles of chlorophyll is formed per mole of sulphate added. In the dark, the yield is slightly greater, being 0.42 moles of chlorophyll per mole of sulphate added.

Discussion

Presumably the pattern of development of chlorosis is distinct with deficiencies of various essential elements which result in chlorosis. In the case of sulphur deficiency, chlorosis develops in two stages. The first phase is due to the development of a differential between the rate of chlorophyll formation and the rate of cell division, chlorophyll synthesis decreasing before cell division. The second phase is due to decomposition of chlorophyll in the cells and is initiated at about the time cell division ceases. Attention should be directed to the fact that both cell division and chlorophyll synthesis do not stop abruptly as the supply of sulphur becomes limiting. There is a gradual decrease in rate. This can be interpreted in two ways. It is conceivable that this is not real but is only an apparent, statistical effect; the individual cells in a culture being of different ages would not all show deficiency symptoms at the same time. Microscopic observation reveals this to be at least partially true. On the other hand, it is possible that the phenomenon is real—that as the concentration of available sulphur decreases to some minimum value, the rate of chlorophyll synthesis, etc., gradually decreases. If this is true, it follows that the rate of cholorphyll synthesis and other metabolic processes are determined, at least partially, by the concentration of sulphate when it becomes limiting. In the author's opinion the true interpretation of the gradual decrease in rate is to be found by combining these two explanations. Assuming that the rate of chlorophyll syn-

thesis and other metabolic processes decrease gradually as sulphate becomes limiting, it follows that the rates of these processes are functions of sulphate concentration under these conditions. In this connection it is significant to note that with a decreasing supply of sulphate there is a differential action upon at least some metabolic processes. More specifically, chlorophyll formation is more sensitive to a deficiency of sulphur than is cell division. Differential responses of this nature could be useful in studies of sulphur metabolism.

Further evidence illustrating a disturbance of cell metabolism in sulphur-deficient cells is the observation that these chlorotic cells contain large quantities of fat, and little or no starch. This fat accumulation also occurs when the cells are grown autotrophically. (Starch is the normal storage product in Chlorella, very little or no fat being found.)

Addition of sulphate to sulphur-deficient cells was found to result in rapid recovery from chlorosis. When sulphate is added in concentrations of 0.5 p.p.m. of S, or greater, the recovery curve showing the formation of chlorophyll can be separated into four phases: (1) a lag period; (2) a period of increase in rate of synthesis to a maximum; (3) a period of exponential increase in chlorophyll at the maximum rate; and (4) a decrease in rate of synthesis to zero or to normal depending upon the amount of sulphate available.

It is conceivable that the initial lag is due to slow penetration of sulphate. This would appear very unlikely. If slow penetration were responsible there should be some direct relation between concentration of sulphate and length of the lag period since, according to Fick's law, the rate of diffusion is directly proportional to the concentration gradient. No such relation was found. If this lag is a real phenomenon, then it is to be inferred that sulphate bears no simple, direct relation to the synthesis of chlorophyll. It is possible that sulphate is metabolized during the lag period, these anabolic processes culminating or being involved in the synthesis of chlorophyll. On the other hand, it is conceivable that free sulphate initiates or activates a series of reactions leading to chlorophyll formation. It would seem that the first of these postulates is more reasonable in view of the stoichiometric relation between sulphate added and chlorophyll formed.

The initial degree of chlorosis—that is, the degree of sulphur starvation—has some bearing on the behavior during the lag period. The effect cannot be stated in any more definite terms because of insufficient data. It should be pointed out, however, that significant decomposition of chlorophyll occurs during the first few hours after addition of sulphate to slightly chlorotic cells.

An interesting phase of the formation of chlorophyll during recovery is the exponential increase in chlorophyll. The slope of the line showing the logarithmic increase in chlorophyll is several times (2.6) greater during recovery than during normal growth in full nutrient solution. It would appear from this that the factor (or factors) normally limiting the rate of

chlorophyll synthesis is not in operation during recovery from chlorosis. It should be pointed out in this connection, that with increased initial chlorosis the rate of logarithmic formation of chlorophyll during recovery was found to be greater. Although several interpretations of the behavior are possible the information available is inadequate to make discussion profitable.

The relation between the amount of sulphate added to a suspension of chlorotic cells and the amount of chlorophyll formed is significant. Summaries of data from several experiments reveal that about 0.34 moles of chlorophyll are formed per mole of sulphate added over a concentration range of 0.01–1.93 p.p.m. of S. This independence of the chlorophyll yield upon concentration of sulphate would indicate complete absorption of sulphate. The existence of this stoichiometric relation would seem to preclude any direct or indirect catalytic action of sulphate.

With greater initial chlorosis the chlorophyll yield is slightly increased. It should be mentioned that the increased yield is the same at different concentrations of sulphate and is equal to the differences in the initial amount of chlorophyll present in the cells. Thus if a given amount of sulphate is added to suspensions of cells of different degrees of chlorosis the maximum chlorophyll content which is found in the different suspensions is the same. An obvious hypothesis to interpret this behavior would be that decomposition products accumulate in the cells when the degree of chlorosis increases due to chlorophyll decomposition and that synthesis of chlorophyll from these products can occur during recovery without utilization of sulphate. This hypothesis must be discarded, however, since more rigid consideration of the data do not lend support.

For many years, it has been known that Chlorella can form chlorophyll when grown in the dark. It has also been recognized that when cultured in the dark on glucose, Chlorella grows much more slowly than in the light. It is interesting to note in this connection that light has very little effect on chlorophyll formation during recovery from sulphur deficiency, the rate of synthesis being only slightly greater in the light. On the other hand, the amount of chlorophyll formed in the dark per mole of sulphate is significantly greater. This increase in yield would seem too great to be explained on the basis of more rapid decomposition in cells in the light. With respect to initiation of cell division during recovery there is a very pronounced effect of light—cell division being many times slower in the dark. It might be inferred that more sulphate is available for the processes resulting in chlorophyll formation when recovery occurs in the dark.

An analysis of the effect of various sulphur compounds on recovery could be used to advantage in studies of sulphur metabolism. While the data presented here which relate to sulphur metabolism are rather meager, brief discussion will be profitable. Since cysteine hydrochloride, dl-methionine, glutathione, thioglycollic acid and potassium thiocyanate were all found to be unavailable for recovery, it would appear that the course of sulphur metabolism does not proceed through one of these compounds. If

it does, then we must assume that the metabolism proceeds in several directions and that these reactions are not readily reversible. An alternative interpretation is impermeability of the cell to these compounds. This would not seem to be the case with cysteine since LUDWIG (9) has shown that it can be used as a source of nitrogen by this same strain of Chlorella.

All the inorganic compounds tested were available for recovery—thiosulphate, pyrosulphate, sulphite, persulphate, and hydrogen sulphide. When sulphur is supplied as H_2S the lag period is slightly longer, the acceleration in chlorophyll synthesis is slightly lower and the maximum rate of synthesis attained is less than when sulphate is supplied. This would lead to the conclusion (if questions of toxicity and rate of penetration are neglected) that sulphur in the form of H_2S must undergo more radical changes than sulphate before participation in metabolism. It is conceivable that oxidation to sulphate takes place. The response to sulphide in the dark is significantly slower than in the light. Possibly the lower oxidation potential in the cells under a condition of darkness results in slower oxidation to sulphate.

Lack of quantitative data on the course of recovery with the other inorganic compounds precludes any postulation of their relation to sulphur metabolism other than that they are assimilable.

Summary

1. Chlorella sp. was grown under controlled conditions in sulphur-deficient cultures. The development of chlorosis and the process of recovery from chlorosis have been analyzed. The chlorophyll deficit of the chlorotic cells has been put on a quantitative basis and expressed as a percentage of the normal chlorophyll content.

2. Chlorosis induced by sulphur deficiency develops in two phases due respectively to: (a) A differential between the rate of cell division and the rate of chlorophyll synthesis as they both decline to zero. This results in a decrease in the amount of chlorophyll per cell before cell division stops. (b) Slow decomposition of chlorophyll in the non-dividing cells.

3. Fat accumulates in the deficient cells (starch is the normal storage product).

4. Addition of sulphate to deficient cells results in rapid recovery from chlorosis. Although chlorophyll synthesis is evident within about 5 hours, cell division does not occur until about 24 hours after addition of sulphate. Chlorophyll formation will occur at a lower concentration of sulphate than will cell division.

5. The curve showing the formation of chlorophyll during recovery can be separated into four stages: (a) A lag period of about five hours between addition of sulphate and synthesis of chlorophyll. (b) A period of acceleration in synthesis of chlorophyll during which the rate of formation increases from zero to a maximum over a period of about five hours. (c) A period of logarithmic increase in chlorophyll at a rate faster than occurs during normal growth in full nutrient solution. (d) A period of decrease in rate of synthesis.

6. The effect of various factors on recovery was studied: light *vs.* darkness; initial degree of chlorosis; concentration of sulphate; sources of sulphur available (organic and inorganic); necessity of oxygen.

7. The chlorophyll yield during recovery is constant over the concentration range 0.01–1.93 p.p.m. of S as sulphate, about 0.34 moles of chlorophyll being formed per mole of sulphate added. It is inferred that complete absorption of sulphate takes place.

The author thanks Professor LEWIS KNUDSON for his interest in the problem and for his criticisms of the manuscript.

CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. EMERSON, R. Relation between maximum rate of photosynthesis and chlorophyll concentration. Jour. Gen. Physiol. **12**: 609–622. 1929.
2. ———, and ARNOLD, W. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.
3. ———, GREEN, L., and WEBB, J. L. Relation between quantity of chlorophyll and capacity for photosynthesis. Plant Physiol. **15**: 311–317. 1940.
4. FLEISCHER, W. The relation between chlorophyll content and rate of photosynthesis. Jour. Gen. Physiol. **18**: 573–597. 1935.
5. HOPKINS, E. F., and WANN, F. B. The effect of the H-ion concentration on the availability of iron for Chlorella sp. Jour. Gen. Physiol. **9**: 205–210. 1925.
6. ———, and ———. Relation of hydrogen-ion to growth of Chlorella and to the availability of iron. Bot. Gaz. **81**: 353–376. 1926.
7. ———, and ———. Further studies on the growth of Chlorella as affected by hydrogen-ion concentration. Bot. Gaz. **83**: 194–201. 1927.
8. KENNEDY, S. R. JR. The influence of magnesium deficiency, chlorophyll concentration, and heat treatments on the rate of photosynthesis of Chlorella. Amer. Jour. Bot. **27**: 68–73. 1940.
9. LUDWIG, C. A. The availability of different forms of nitrogen to a green alga. Amer. Jour. Bot. **25**: 448–458. 1938.
10. PEARSALL, W. H., and LOOSE, L. The growth of *Chlorella vulgaris* in pure culture. Proc. Roy. Soc. (London) Ser. B. **121**: 451–501. 1937.
11. SCHERTZ, F. M. The preparation of chlorophyll. Plant Physiol. **3**: 487–497. 1928.
12. WANN, F. B. The fixation of free nitrogen by green plants. Amer. Jour. Bot. **8**: 1–29. 1921.

STRUCTURE AND COMPOSITION OF CITRUS LEAVES AFFECTED WITH MESOPHYLL COLLAPSE[1]

F. M. TURRELL, V. P. SOKOLOFF, AND L. J. KLOTZ

(WITH SIX FIGURES)

Introduction

A pathological condition not infrequently observed in leaves of orange trees as yellow, translucent, sunken areas, has been called "mesophyll collapse" by FAWCETT (**6**). Neither bacteria nor fungi seem to initiate this disorder of the leaves, though they may invade the weakened and collapsed tissues secondarily. The disorder has therefore been regarded as a physiological disturbance caused by insects and by edaphic and climatic factors, or by edaphic or climatic factors alone.

FAWCETT (**6**) first found mesophyll collapse in the coastal districts of southern California. In a series of papers, FAWCETT *et al.* outlined the distribution of mesophyll collapse in South America. They found it in the coastal areas of the state of Rio de Janeiro, Brazil (**11**), but did not find it two hundred miles in the interior, in the state of Minas Geraes (**12**). It was also found fairly near the coast, in the states of Pernambuco (**7**) and Bahia (**8**), and in Sao Paulo as far as 40 miles inland and at elevations as high as 2,500 feet above sea level (**8**). None was noted in the Pitangueires area, some two hundred miles from the sea coast; in the state of Rio Grande do Sul, however, it was found near the coast, at Montenegro (**8**). It was noted at Uruguayana, somewhat inland, near the Uruguay River (**8**), as well as at Concordia, Argentina, also near the Uruguay River. These investigators, however, found no mesophyll collapse in any other parts of Argentina (**9**). In Paraguay, mesophyll collapse was found at Asuncion, near the Paraguay River (**10**).

The writers have made observations in North America and have noted the occurrence of mesophyll collapse in the coastal districts of the United States and Mexico. We have found it in the coastal districts of Florida, near Vero Beach, and in the coastal valleys of California. It has been observed in Arizona[2] and it probably exists, also, in some parts of Texas near the gulf. Our survey in California has shown that the disorder is present in Ventura, Santa Barbara, Los Angeles, San Bernardino, Riverside, Orange, and San Diego counties. Its general distribution in California is shown in figure 1.

This leaf condition is most readily recognized when the mature leaves are examined by transmitted light. It will then be observed that yellow, translucent areas lacking chlorophyll occur principally in the central portion of the blade, on one or both sides of the midrib (plate I, fig. 2). By reflected

[1] Paper no. 484, University of California Citrus Experiment Station, Riverside, California.

[2] Observation reported by DR. R. B. STREETS, Associate Plant Pathologist, University of Arizona, Tucson, in letter to the senior author, dated March 1, 1943.

light these areas on the lower surface of the leaf appear grayish and somewhat sunken below the normal leaf surface. Necrosis or browning of the translucent areas may also occur. The leaves, however, are otherwise apparently normal, as are the twigs on which they are carried. So far, the symptoms have been observed on Valencia and Washington Navel oranges, but not on lemons.

The disorder is of interest because it represents one of the foliar symptoms for which explanations have long been sought. HAAS (**14**) has suggested that a water deficit during the hot summer months may be involved,

FIG. 1. Hatched portion indicates California citrus areas in which mesophyll collapse in orange leaves is prevalent.

though mesophyll collapse is seen in very young trees under conditions where no great stress on the transpiration system has occurred. Trees growing under optimum soil- and air-moisture conditions and subjected to occasional severe water deficits, generally show the greatest incidence of mesophyll collapse, while trees continually subjected to water deficit are least affected. In groves observed by us, leaves showing the disorder were found only on the north side of the affected tree, or they were generally distributed around the tree.

The prevailing opinion is that the necrosis following infestation with the citrus red mite, or red spider, *Paratetranychus citri* (McG.), is a forerunner or an accompaniment of mesophyll collapse. In fact, the distribution of citrus red mite in California, as shown by QUAYLE (**21**), is approximately

the same as that of mesophyll collapse. Many trees not infested with citrus red mite are bady affected with mesophyll collapse, however.

The purpose of the present paper is to report studies made during 1941 and 1942 on the structure and composition of leaves of citrus trees affected with mesophyll collapse in southern California.

Materials and methods

For Ca, Mg, Na, K, Cl, and certain ash analyses, leaves were collected near Claremont, California, in early June and in late July, from Washington Navel orange trees growing on light sandy soil (Ramona loam), the pH of which, at field capacity, was 4.80 to 6.01 at the 1-foot level. A second sample of leaves for S and certain ash analyses was collected in August from the Washington Navel orange trees at Claremont and from Valencia orange trees at San Juan Capistrano. The Valencia orange trees were growing on heavy-textured Montmorillonitic yellow-clay soil (Diablo adobe clay), which had a pH of 6.15 to 6.39 at field capacity. In October, a third sample of leaves was collected from the San Juan Capistrano grove for S, P, and ash analyses.

Samples of injured and normal leaf tissue, of equal area, were excised from the leaves with a cork borer. For every sample of injured tissue, a sample of normal tissue was excised from a similar position on the blade, on the opposite side of the midrib.

Samples of Washington Navel orange leaf tissue, for sap analyses, were autoclaved 15 minutes at 15 lb. per square inch and pressed at 16,000 lb. per square inch. The sap and press cake analyses for Ca, Mg, Na, K, and Cl were made on 100 grams of sap and on 100 grams of press cake dried at 70° C., respectively. Methods of the United States Department of Agriculture Bureau of Plant Industry, Division of Irrigation Agriculture, Rubidoux Laboratory, located at Riverside, California, were used.

Analyses for total S as SO_4 were made according to the methods of the Association of Official Agricultural Chemists. Analyses for P as PO_4 were made according to methods of the Rubidoux Laboratory, using $(NH_4)_3PO_4 \cdot 12\ MoO_3$. All leaves for these analyses were samples as previously described. The samples were heated for 2 hours at 110° C. to inactivate the enzymes and were then dehydrated at 65° C., *in vacuo,* until the material attained constant weight.

Samples of affected and healthy areas of leaves collected in August from the same groves as the material for chemical analyses, were killed in formalin acetic acid alcohol and prepared for microscopical examination by the paraffin method. Sections 12 μ in thickness were stained with Delafield's hematoxylin and safranin, and permanent mounts were made.

Results

Results of a brief study of the distribution of collapsed areas in affected leaves of Washington Navel and Valencia orange are shown in table I. For this study, each leaf was divided into two portions, the outer or marginal

portion and the inner or middle portion; the collapsed areas at proximal, central, and distal locations in these portions were counted. It was clear that the incidence of the disorder occurred principally in the central portion of the leaves.

Of the Washington Navel orange leaves which exhibited mesophyll collapse (table I), 17.2 per cent. were small, 54.4 per cent. were medium, and 28.4 per cent. were large. Of the Valencia orange leaves which exhibited mesophyll collapse, 31.7 per cent. were small, 40.0 per cent. were medium, and 28.3 per cent. were large. This is what would be expected from the normal size distribution of orange leaves. In the Washington Navel orange leaves, 68.7 per cent. of the translucent areas contained necrotic areas (brown spots); in the Valencia orange leaves, 40.3 per cent.

TABLE I

DISTRIBUTION OF AREAS AFFECTED WITH MESOPHYLL COLLAPSE IN ORANGE LEAVES, EXPRESSED IN PERCENTAGE OF TOTAL NUMBER OF COLLAPSED AREAS

LOCATION	WASHINGTON NAVEL		VALENCIA	
	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION
	%	%	%	%
Proximal	4.0	8.0	9.3	16.3
Central	18.7	42.0	13.9	33.4
Distal	9.3	18.0	10.8	16.3

From a comparative histological examination of normal green leaf tissue and of affected leaf tissue, it was evident that the injured tissue contained collapsed spongy mesophyll cells (plate I, figs. 3–6). Until the affected areas dried, the palisade tissue was apparently normal (plate I, figs, 3–4). Intercellular space was reduced in the abnormal tissue, owing to the collapse of much of the spongy mesophyll and the enlargement of certain spongy mesophyll cells which had not collapsed. The diameters of the enlarged cells averaged 35 μ, whereas the diameters of normal cells averaged 18 μ. The walls of the enlarged cells had a maximum thickness of 2 μ; walls of normal sponge cells had a maximum thickness of 0.8 μ. Collapsed and injured tissue stained deeply with safranin, as shown by the dark regions in figure 4. The disorder was not confined to one vein islet, but included several to many.

Only rarely was the palisade tissue abnormal above the abnormal spongy mesophyll cells. As shown in plate I, figure 6, the palisade cells are shortened and not elongated. This suggests that, in addition to hypertrophy and collapse in the sponge tissue, on rare occasion hyperplasia may be observed in the palisade tissue, a condition reminiscent of boron deficiency. The palisade mesophyll directly above the affected spongy mesophyll is generally normal, however. This is confirmed by examination of tangential sections through palisade tissue above normal spongy mesophyll and above abnormal spongy mesophyll.

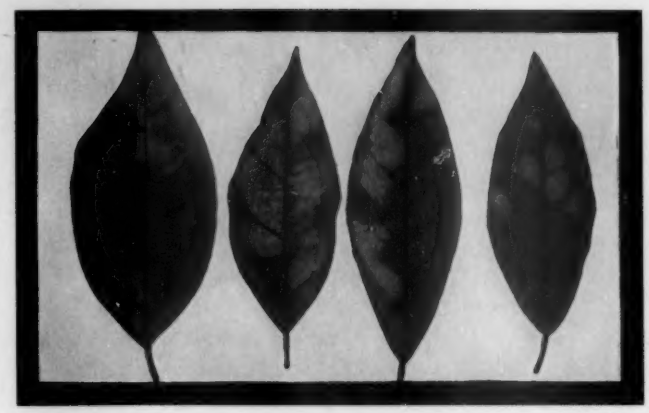
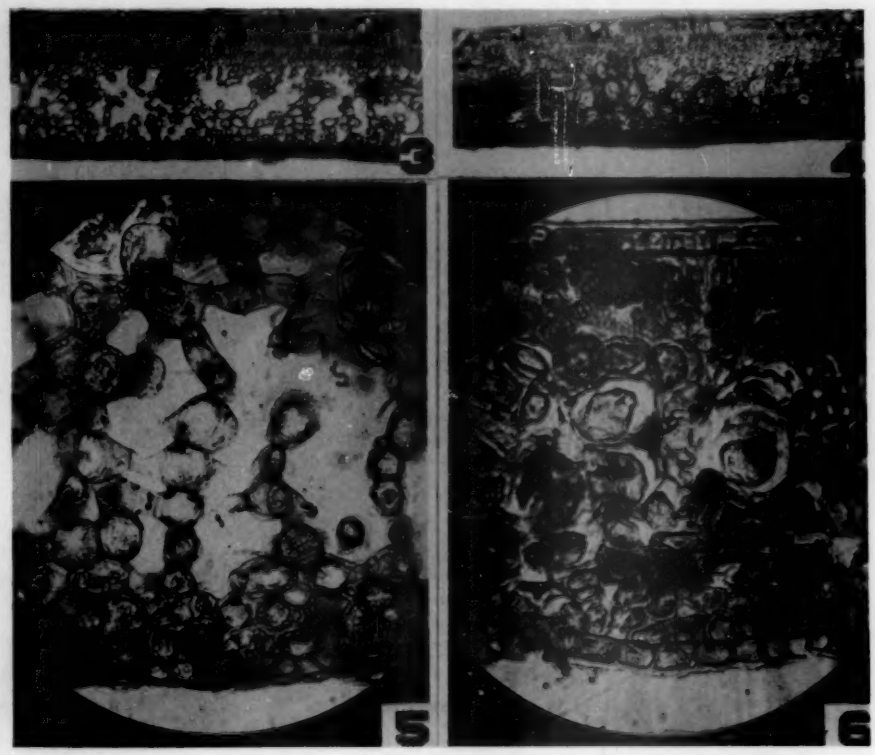


FIG. 2. Mesophyll collapse of sweet orange leaves photographed by transmitted light. (From color plate 27, Klotz and Fawcett, [19]).



Photomicrographs of transverse sections of Washington Navel orange leaves.

FIG. 3. Section through normal portion of leaf showing greater leaf thickness and large intercellular space. x 64.

FIG. 4. Section through abnormal portion of leaf showing reduced leaf thickness, collapsed mesophyll and abnormally enlarged mesophyll cells. x 64.

FIG. 5. Same section as Fig. 3. x 287.

FIG. 6. Same section as Fig. 4. x 287.

TABLE II

SULPHATE AND CARBONATE ASH IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON NAVEL AND VALENCIA ORANGE, EXPRESSED IN GRAMS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	SO_4 ASH	CO_2 ASH
		WASHINGTON NAVEL	
		gm.	*gm.*
1	Collapsed	14.8	11.1
1c	Normal	16.8	11.4
2	Collapsed	17.8	11.2
2c	Normal	18.3	11.5
3	Collapsed	17.7	11.6
3c	Normal	18.9	11.2
4	Collapsed	18.4	11.3
4c	Normal	19.6	11.2
		VALENCIA	
7	Collapsed	15.8	8.9
7c	Normal	17.8	13.5

The normal spongy mesophyll, as seen in tangential section, contains intercellular space. The abnormal spongy mesophyll, prior to collapse, shows hypertrophy of certain sponge cells so abutting that no intercellular space is visible.

While normal leaves vary greatly in thickness, sections of the same leaf at loci where normal tissue is found, and where mesophyll collapse is apparent, differ greatly. The averages of numerous measurements indicated that, in normal tissue, the leaf thickness was 283 μ, palisade thickness was 91 μ, and sponge thickness was 183 μ; while in abnormal tissue, the leaf thickness was 255 μ, palisade thickness was 87 μ, and sponge thickness was 153 μ. It was of especial interest to note that the cuticle was intact in all sections. Chloroplasts seemed to be fewer in number in the enlarged spongy mesophyll cells than in the normal.

TABLE III

DISTRIBUTION OF ALKALINE-EARTH AND ALKALI BASES AND OF CHLORIDE BETWEEN EXPRESSED SAP AND PRESS CAKE OF COLLAPSED AND OF NORMAL LEAF TISSUE OF WASHINGTON NAVEL ORANGE, EXPRESSED AS MILLIEQUIVALENTS PER 100 GRAMS OF SAP OR DRY SUBSTANCE, RESPECTIVELY

SAMPLE	LEAF TISSUE	EXPRESSED SAP					PRESS CAKE				
		Ca	Mg	K	Na	Cl	Ca	Mg	K	Na	Cl
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
3	Collapsed	44	4	12	3	6	234	29	13	14	1
3c	Normal	68	1	10	1	3	256	11	8	5	1
4	Collapsed	44	3	7	3	3	236	22	8	6	1
4c	Normal	91	2	5	1	1	278	14	7	3	2

Chemical analyses indicate a number of differences between collapsed and normal tissue. As shown in table II, the sulphate ash in the dry matter of collapsed tissue was in every case lower than that in the dry matter of normal tissue. On the other hand, the carbonate ash was about the same in both normal and collapsed tissue.

The Ca content of the sap of collapsed tissue was lower than that of the sap of normal tissue (table III) but the Mg, K, Na, and Cl contents were higher in the sap of collapsed tissue than in that of normal tissue (table III).

The Ca content of the press cake of collapsed tissue, also, was lower than that of normal tissue (table III); and the Mg, K, and Na contents were higher in the press cake of collapsed tissue than in that of normal tissue (table III). There was no significant difference in the Cl content of the press cake from normal and collapsed tissue (table III).

TABLE IV

ALKALINE-EARTH AND ALKALI BASES IN COLLAPSED AND IN NORMAL LEAF TISSUE OF WASHINGTON NAVEL ORANGE, EXPRESSED IN MILLIEQUIVALENTS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	Ca	Mg	K	Na	TOTAL Ca, Mg, K, AND Na
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
1	Collapsed	177	19	63	5	264
1c	Normal	229	11	32	1	273
2	Collapsed	230	22	40	3	295
2c	Normal	258	13	23	3	297
3	Collapsed	245	30	15	15	305
3c	Normal	297	12	14	5	328
4	Collapsed	277	25	14	9	325
4c	Normal	382	15	13	4	414

Analyses of unexpressed normal and collapsed leaf tissues for alkaline-earth and alkali bases are shown in table IV. The analyses of samples 1, 1c, 2, and 2c are averages of three separate examinations; namely, analysis of plant ash, the sum of 30 per cent. C_2H_5OH-soluble and -insoluble fractions, and the sum of fractions soluble and insoluble in 0.05 N $BaCl_2$ in 30 per cent. C_2H_5OH. Analyses of samples 3, 3c, 4, and 4c are summations of analyses of expressed sap and of press cake. The results shown in table IV are in agreement with the direction of magnitudes of the cation contents in the sap and press cake analyses shown in table III; that is, the Ca is lower in the collapsed tissue than in the normal tissue, and the other cations are higher. The Na content of sample 2 presents the only exception: it was the same as that of the normal tissue of sample 2c.

Analyses made of the unexpressed normal and collapsed leaf tissues for total S as SO_4 and P as PO_4 are shown in table V. In samples 5 and 5c from Washington Navel orange leaves, and 6 and 6c from Valencia orange leaves, the normal tissue contained more S than the collapsed tissue. In the Va-

lencia leaves of samples 7, 7c, 8, and 8c, however, the S content of the collapsed tissue was higher than that of the normal tissue. In all cases, the P values were higher in the collapsed tissue than in the normal tissue. Semiquantitative microanalyses in samples 5, 5c, 6, and 6c indicated larger concentrations of P in collapsed tissue. The percentage of moisture in collapsed and normal samples of leaf tissue are also shown in table V. The normal leaf tissue of Washington Navel orange and of Valencia orange contained a higher percentage of moisture than the collapsed tissue. The pH of normal leaf sap was 5.42, which was slightly, but not significantly, higher than the pH of sap from collapsed leaf tissue, which was 5.37.

TABLE V

PERCENTAGE MOISTURE, TOTAL SULPHUR, AND TOTAL PHOSPHORUS IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON NAVEL AND VALENCIA ORANGE, EXPRESSED IN MILLIGRAM ATOMS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	MOISTURE	S	P
		WASHINGTON NAVEL		
		mg. atom.	*mg. atom.*	*mg. atom.*
5	Collapsed	47.10	7.3*	3.8
5c	Normal	48.16	8.3*	3.0
		VALENCIA		
6	Collapsed	41.87	9.2*	3.5
6c	Normal	42.83	12.1*	3.1
7	Collapsed	.....	23.5	2.9
7c	Normal	.....	20.5	2.3
8	Collapsed	.....	28.2	2.8
8c	Normal	.....	23.6	1.7

* The authors wish to thank PROFESSOR A. J. HAAGEN-SMIT, California Institute of Technology, for the microanalyses reported here.

Comparison of our leaf analyses with those of other workers dealing with various nutrient deficiencies and excesses, indicated that the principal differences between their results and ours lay in the high Mg values obtained by us. Consideration of the soil types where mesophyll collapse occurred in California, suggested that Mg^{++} might be associated with the disorder.

Several experiments were run with potted sour-orange seedlings, which were watered with 50 e.p.m. of $MgSO_4$ solution for two weeks and then leached with distilled water. After two days of the latter treatment, leaf symptoms of mesophyll collapse developed. The leaves were sectioned and examined. Similarity to mesophyll collapse was evident.

Discussion

Ignited residue weighed as carbonate ash is obtained at the relatively low temperatures of dull-red heat. It contains, chiefly, combinations of carbonates, oxides, and phosphates of the alkaline-earth and alkali bases; small amounts of Si, SO_4, and Cl; and other quantitatively minor constituents.

Organic compounds of the bases, such as $CaC_2O_4 \cdot H_2O$, for example, and nitrates are converted into carbonates or oxides in the course of the combustion.

Sulphate ash, on the other hand, is obtained at much higher temperatures. The plant material, prior to the combustion, is treated with H_2SO_4. Under such conditions the ignited residue consists largely of sulphates and phosphates. As the equivalent combining weight of SO_4 is higher than that of CO_3 or O, correspondingly higher figures for the SO_4 ash are obtained, all other things being equal.

In view of these considerations, it is possible to interpret the results shown in table II in the following manner. Organic compounds of the bases are less prominent in the collapsed tissue than in the normal tissue, as evidenced by the consistently smaller differences between the SO_4 and the CO_3 ash of the collapsed tissue. This conclusion is also supported by the fact that SO_4 ash is higher in the normal tissue than in the collapsed tissue, while carbonate ash, in all samples except those of Valencia orange, is nearly identical in the two tissues. Furthermore, were the CO_3 ash residues largely identical in kind, the trends in the SO_4 ash would not have become manifest. One may infer, therefore, that, while some quantitatively important "acidic" constituents of the CO_3 ash of collapsed tissue are similar in their equivalent combining weight to those of the CO_3 ash of normal tissue, they are not converted to SO_4 in the course of the SO_4 ash determination. Tertiary PO_4 would be the most likely type of substance responsible for this difference. If it is assumed that the observed difference is due to tertiary PO_4, the collapsed tissue would be expected to contain more PO_4 than the normal tissue, but less of the organic compounds of the alkaline-earth and alkali bases. Actually, analyses of the tissue for PO_4 showed that this was the case.

Relative amounts of Ca in the sap phase of collapsed tissue are much lower than in the corresponding samples from normal tissue. The ratio of Ca to the alkali bases, for example, is 3 and 4 in the sap of collapsed tissue as against 6 and 15 in that of normal tissue, for samples 3 and 4 and 3c and 4c, respectively. In the press cake analyses, similar trends are also manifest; but, recognizing that variable amounts of sap are retained in the press cake against a pressure of 16,000 lb. per square inch, we have not relied on the press cake analyses alone.

Hydration of colloids contained in the leaf tissue is most probably influenced by the kind of cations combined or associated with the colloids. It is reasonable to infer that, all other things being equal, any increase in the proportion of monovalent cations, that is, K^+ and Na^+, in the liquid phase in equilibrium with the colloidal surfaces, would produce corresponding changes in the cations adsorbed by the colloids; whereupon the extent of hydration of the latter would in all probability be increased. In fact, STUEWER (**23**) showed that, in aqueous solution, pectic acid is 21 per cent. hydrated; pectin, 25; calcium pectate, 35; and sodium pectate, 38. Such an increase in hydration would also be brought about by the removal of Ca^{++} by replacement with monovalent cation.

Precipitation or translocation of Ca^{++} would be expected after replacement, and our analyses suggest the latter possibility rather than the former. Any increase in amounts of cations other than Ca^{++}, would tend to increase the mobility of Ca^{++} in the plant tissue, whether this Ca is in the adsorbed or in the precipitated form.

STUEWER (**23**), as a result of his experiments, states that the sodium salts of pectin are highly dissociated, that the salts of divalent metals are considerably less dissociated, and that the acid is still less dissociated. These results are in agreement with the reported facts that sodium salts hinder jelling, while calcium chloride promotes it. In our own studies, despite variations in the absolute amounts of the bases and in every comparison of collapsed tissue with corresponding normal tissue, Mg, K, and Na were always higher in the collapsed tissue than in the normal tissue, while Ca was always lower. This suggests that the weakening of the gel structure in the spongy mesophyll cells may be caused by the high concentrations of Mg^{++}, K^{+}, and Na^{+}, and the low concentrations of Ca^{++}, so that in irregularly occurring periods of unusual transpirational stress, collapse of the cells results.

The work of KERTESZ *et al.* (**18**) seems to support this interpretation of results. They found that Ca salts added to canned tomatoes result in the better retention of the original firmness of the fruit. It was postulated by KERTESZ (**17**) that the addition of Na or K salt accomplished just the opposite of the Ca effect, inasmuch as Ca is removed from the tissue by an exchange of ions. This reaction was confirmed by GREENLEAF, according to KERTESZ *et al.* (**18**). GREENLEAF (**13**) has further shown that the Ca salt improves the texture of the canned product by forming calcium pectate.

The findings of STEWARD, STOUT, and PRESTON (**22**), in their respiration studies, indicate that K^{+} increases the mobility of pectin. They found that KBr at high oxygen tension, through the action of K^{+} on Ca^{++}, caused the transfer of a uronic acid from potato disks to blotting papers; this, when calculated as pectin, accounted for the concomitant loss of carbon on their balance sheets. It also seems possible that such colloids as proteins might act in a similar manner under the influence of K^{+} or Na^{+}.

The fact that normal leaf tissue has a higher percentage of moisture than tissue containing collapsed spongy mesophyll cells, may be interpreted as support of the explanation of the mechanism of collapse, since weakening of the gel structure to the extent where collapse would occur would be followed by a loss in water and necrosis. This process is commonly observed as a result of freeze injury in citrus fruits and affects entire vesicles.

While these facts suggest the mechanism of the collapse of the spongy mesophyll, they do not contain any suggestion as to the mechanism of the enlargement of certain of the spongy mesophyll cells found among the collapsed cells.

The theory that the citrus red mite, or red spider, may be responsible for mesophyll collapse seems to offer an explanation of the enlarged cells, since the effect of certain indole derivatives and excreta from insects is known to

cause intumescences in leaves (**20**). Mesophyll collapse may be abundant, however, where red spider infestations are light; and in greenhouse studies with red spider, we failed to produce symptoms of mesophyll collapse. Macroscopic examination of the surface of certain leaves from the field, exhibiting mesophyll collapse, failed to show symptoms of red spider injury, such as silvering of the upper surface of the leaf. Microscopic examination in cross section failed to show spots in the palisade parenchyma lacking chlorophyll, which we have found in silvered leaves having heavy infestations of red spider. We have therefore questioned the red spider explanation of mesophyll collapse, despite the similar distribution of the two.

HAAS and THOMAS (**15**) have shown that in leaves from Lisbon lemon trees, severe injury is correlated with a high S content ranging from 0.68 to 1.10 per cent. of the dry matter. In the field, SO_4 injury occurred where $NaNO_3$ was absent in fertilizers containing blood and bone meal and K_2SO_4; or blood, bone meal, and K_2SO_4; or impure $CaH_4(PO_4)_2$ (superphosphate), alone or in combination with organic fertilizers, but without $NaNO_3$. The SO_4 content of mesophyll-collapsed tissue, however, is about normal. Values agree well with those reported by KELLEY and CUMMINS (**16**) for normal leaves and with those reported by HAAS and THOMAS (**15**) for uninjured leaves. But our values are much lower than those reported by HAAS and THOMAS for leaves showing severe SO_4 injury.

Analyses of CHAPMAN and BROWN (**2**) on leaves of P-deficient navel-orange trees, show a disturbed ionic balance in the leaf tissue similar to that found by the authors in mesophyll collapse, except that the P content was low in their material and high in ours. The values of CHAPMAN and BROWN (**3**) for S-deficient plants, as compared with healthy plants, agree with our values for collapsed tissue of mature leaves, as compared with normal leaf tissue, except in S content, where our results were variable. The values of CHAPMAN and LIEBIG (**4**) for high-SO_4 plants at various nitrogen levels are similar to our results for Ca, K, and P; but Mg is low and S is high for high-SO_4, while in mesophyll-collapsed tissue, Mg is high and S is variable. Only a few points of agreement were noted between their chloride series and our results.

EATON (**5**) grew eight crops in nutrient media containing 50 and 150 milliequivalents of Cl and 50, 150, and 250 milliequivalents of SO_4 per liter. His sap analyses of these crops for Ca, Mg, K, and Na, as well as the sum of the cations in several instances, show the same trend for high SO_4 plants, as compared with control, as our results show for collapsed tissue as compared with normal tissue.

From the preceding comparisons, it may be surmised that mesophyll collapse is related chemically to an unbalance in the ionic constituents of the plant. It is possible that this unbalance may be due to excesses or deficiencies in soil constituents.

Morphologically, the appearance of mesophyll collapse may resemble the external leaf symptoms sometimes obtained in water culture in B deficiency

or in cultures at high nitrate levels, using $Ca(NO_3)_2$. Certain leaves showing symptoms of mesophyll collapse were obtained from such cultures[3] for histological examination. None of these leaves contained collapsed spongy mesophyll cells, but they showed distinct histological characteristics peculiar to the two types of cultures from which they were obtained. CAMP[4] states that he has produced symptoms of mesophyll collapse by treating dry citrus trees with excesses of KNO_3, or with $Ca(NO_3)_2$. BAIN and CHAPMAN (1) produced similar symptoms in grapefruit cultures, using $Ca(NO_3)_2$ in combination with waterlogged soil.

Further work will be necessary to determine whether one or several types of ionic unbalance may bring about typical external and internal symptoms of mesophyll collapse.

Summary

Mesophyll collapse apparently occurs in leaves of orange trees in the coastal regions of all southern California orange districts. It is confined largely to leaves of orange and has seldom been seen on those of lemon. Leaves exhibiting mesophyll collapse have chlorotic, translucent, water-soaked-appearing areas, principally in the central portion of the leaf blade, although collapse symptoms may occur anywhere in the lamina; brown necrotic spots may develop in the translucent areas. Histological examination of affected leaf tissue shows enlarged sponge cells interspersed with collapsed sponge cells, reduced intercellular spaces, and chlorophyll-depleted spongy mesophyll cells.

Ca was invariably found to be lower in collapsed tissue than in normal tissue, while K, Mg, Na, Cl, and P were somewhat higher than in normal tissue. The ratio of Ca to K was accordingly lower in the collapsed tissue than in the normal tissue. Distribution of the alkaline-earth and alkali bases between the liquid (sap) and the solid phases of autoclaved leaf tissue likewise showed similar differences in absolute and in relative amounts of Ca and K. No consistent difference in the S content of collapsed and normal tissue was found. Consistently lower percentages of SO_4 ash were found in the collapsed tissue than in the normal tissue, while the CO_3 ash was the same in both normal and collapsed tissue.

The writers are indebted to Prof. H. S. FAWCETT for his assistance and cooperation throughout the work, and to PROFESSORS E. T. BARTHOLOMEW, W. H. CHANDLER, R. W. HODGSON, A. M. BOYCE, and H. D. CHAPMAN for critical reading of the manuscript.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BAIN, F. M., and CHAPMAN, H. D. Nitrate fertilizer additions to waterlogged soils in relation to oxygen deficiency. Soil Sci. **50**: 357–367. 1940.

[3] Through the courtesy of DR. A. R. C. HAAS.

[4] CAMP, DR. A. F. In a letter to PROF. H. S. FAWCETT, dated January 27, 1943.

2. CHAPMAN, H. D., and BROWN, S. M. The effects of phosphorus deficiency on citrus. Hilgardia **14**: 161–181. 1941.
3. ————, and ————. The effects of sulfur deficiency on citrus. Hilgardia **14**: 183–201. 1941.
4. ————, and LIEBIG, G. F., JR. Nitrate concentration and ion balance in relation to citrus nutrition. Hilgardia **13**: 141–173. 1940.
5. EATON, FRANK M. Toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.
6. FAWCETT, H. S. Citrus diseases and their control. 2nd ed. McGraw-Hill Book Company. New York. 1936.
7. ————, and BITANCOURT, A. A. As doenças dos citrus no Estado de Pernambuco. Boletim da Secretaria de Agricultura, Industria e Comercio (Pernambuco, Brasil) **2**: 317–326. 1937.
8. ————, and ————. Relatorio sobre as doenças dos citrus nos Estados de Pernambuco, Bahia, São Paulo e Rio Grande do Sul. Rodriguesia **3**: 213–236. 1937.
9. ————, and ————. Observaciones sobre las enfermedades de los citrus en el Uruguay. Revista de la Associación do Ingenieros Agronomos (Montevidea, Uruguay) **12**: 3–8. 1940.
10. ————, and ————. Observações sobre as doenças dos citrus no Paraguay. Biologico **6**: 289–296. 1940.
11. ————, GRILLO, H. V. S., and BITANCOURT, A. A. Relatorio sobre as doenças dos citrus no Districto Federal, Estado do Rio de Janeiro e Minas Geraes. I. Districto Federal e Estudo do Rio de Janeiro. Rodriguesia **2**: 329–340. 1936.
12. ————, and MÜLLER, A. S. Relatorio sobre as doenças dos citrus no Districto Federal, Estado do Rio de Janeiro e Minas Geraes. II. Estado de Minas Geraes. Rodriguesia **2**: 340–344. 1936.
13. GREENLEAF, C. A. Suggestions on use of calcium chloride in canned tomatoes. Canner **91**: 11–12. 1940.
14. HAAS, A. R. C. Mesophyll collapse of citrus leaves. California Citrog. **22**: 114. 1937.
15. ————, and THOMAS, E. E. Effect of sulfate on lemon leaves. Bot. Gaz. **86**: 345–354. 1928.
16. KELLEY, W. P., and CUMMINS, A. B. Composition of normal and mottled citrus leaves. Jour. Agr. Res. **20**: 161–191. 1920.
17. KERTESZ, Z. I. The effect of calcium on plant tissues. Canner **88**: 26–27. 1939.
18. ————, TOLMAN, T. G., LOCONTI, J. D., and RUYLE, E. H. The use of calcium in the commercial canning of whole tomatoes. New York Agr. Exp. Sta. Tech. Bull. 252. 1940.
19. KLOTZ, L. J., and FAWCETT, H. S. Color handbook of citrus diseases. University of California Press, Berkeley and Los Angeles. 1941.

20. LaRue, Carl D. The rôle of auxin in the development of intumescences on poplar leaves; in the production of cell outgrowths in the tunnels of leaf-miners; and in the leaf-fall in Coleus. Amer. Jour. Bot. (Abstract) **22**: 908. 1935.
21. Quayle, Henry J. Insects of citrus and other subtropical fruits. Comstock Pub. Co. Ithaca, New York. 1941.
22. Steward, F. C., Stout, P. R., and Preston, C. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. Plant Physiol. **15**: 409–447. 1940.
23. Stuewer, Reinhold F. The hydration and physicochemical properties of pectin and its derivatives. Jour. Phys. Chem. **42**: 305–315. 1938.

RADIANT ENERGY NOMENCLATURE[1]

ROBERT B. WITHROW

Introduction

This report is presented in an endeavor to promote among plant physiologists a more consistent and uniform usage of nomenclature pertaining to radiant energy. The committee has tried to assemble the most authoritative and useful terminology recommendations in a readily available form. No endeavor has been made, however, to present a complete discourse on radiant energy nomenclature since it is considered better to avoid the introduction of terms and relationships which are not likely to be used by plant physiologists.

The nomenclature committees of the Optical Society of America and of the Illuminating Engineering Society have been instrumental in bringing about the standardization of radiant energy terminology (**3, 4, 5, 6, 7, 8, 9, 10, 11**). The present paper is based upon the latest official reports of these two societies (**5, 6, 8**). Definitions of terms not covered in these reports have been taken from other authoritative sources (**1, 2, 12, 13**).

The Physical Methods Committee has found it difficult to decide which terms would be the more useful for plant physiologists where there is disagreement between the terminology recommended by the Optical Society and that recommended by the Illuminating Engineering Society. It finally was decided to give preference to the terms recommended by the Optical Society but to include in parentheses the equivalent terms of the Illuminating Engineering Society. It should be noted that the last report of the Illuminating Engineering Society Committee (**5**) has been accepted by the American Standards Association and probably will be followed rather closely in the engineering fields. The latest published report of the Optical Society (**8**) is preliminary in nature but a final report which includes certain revisions is being prepared for publication. The Physical Methods Committee was informed of the revisions to be made in terms to be included in the present report and these revisions have been taken into account in the preparation of this paper.

Radiometric and photometric terms

Table I presents the recommended system of nomenclature for radiometric and photometric terms. It will be noted that for each radiometric term, there is a corresponding photometric term. *Photometry* is considered as a special case of *radiometry* in which the radiometer or detector of radiant energy is the human eye or a selective detector employed as a substitute for the eye. The plant physiologist seldom is justified in using photometric

[1] A report of the Physical Methods Committee of the American Society of Plant Physiologists. Membership: R. B. WITHROW, Chairman, S. T. DEXTER, K. C. HAMNER, and B. S. MEYER.

TABLE I

RADIOMETRIC AND PHOTOMETRIC NOMENCLATURE AND UNITS

RADIOMETRIC			PHOTOMETRIC		
TERM*	SYMBOL AND DEFINING EQUATION	UNITS	TERM	SYMBOL AND DEFINING EQUATION	UNITS
Radiation			Lumination (Illumination)		
Radiator, source of radiant energy			Luminator (Lamp, Illuminant)		
Radiant energy	U	erg, joule, calorie	Luminous energy (Light)	$Q = Fdt$	lumerg, lumen hour
Spectral radiant energy	$U_\lambda = dU/d\lambda$				
Radiant density	$u = dU/dv$	erg/cm³	Luminous density	$q = dQ/dv$	lumerg/cm³
Radiant flux	$P = dU/dt$	erg/sec, watt	Luminous flux	$F = dQ/dt$	lumen, lumerg/sec
Radiant emittance (Radiancy)	$W = dP/da$	watt/cm²	Luminous emittance	$L = dF/da$	lumen/cm²
Radiant intensity	$J = dP/d\omega$	watt/steradian	Luminous intensity (Candlepower)	$I = dF/d\omega$	candle
Radiance (Steradiancy)	$N = \frac{dP}{d\omega(da \cos\theta)}$	$\frac{\text{watt/steradian}}{\text{cm}^2}$	Luminance (Brightness)	$B = \frac{dF}{d\omega(da \cos\theta)}$	candle/cm², stilb
Irradiation			Illumination		
Irradiance (Irradiancy)	$H = dP/da$	watt/cm²	Illuminance (Illumination)	$E = dF/da$	footcandle, lux, phot
Zero level radiant energy	$U_{\lambda_1}^{\lambda_2} = O$		Dark, zero level luminous energy	$Q = O$	
Radiometry			Photometry		
Spectroradiometry			Spectrophotometry		

* Where there is disagreement between the terms of the Optical Society of America and those of the Illuminating Engineering Society, the O.S.A. terms are given first and the I.E.S. terms follow in parentheses.

terms in dealing with physiological problems since the radiant energy is used in most instances to irradiate plants or non-living physical or chemical systems. Under these circumstances, the radiant energy is not being used for visual purposes. Photometric terms may be used logically in cases, however, where the eye is involved as the detector of the radiant energy, as in the use of visual optical instruments in general and in specifying conditions concerning the visual inspection of objects. As will be discussed later, photometric units may be used as units of irradiance under those conditions where the eye or another detector of similar spectral sensitivity is used as the selective radiometer.

Photo- has been used extensively as a prefix in the restricted sense to mean light and in the more general sense as synonymous with radiant energy. Common uses with the latter connotation are photograph, photochemistry, and photon. Many such terms as these have become so well established that there is little value in attempting to change their prefix to the more logical one of *radi-*.

Radiant energy is that form of energy which is propagated through space in the form of electromagnetic waves. It may be specified in terms of the spectral region involved such as visible radiant energy, ultraviolet radiant energy, or X-radiant energy (X-rays). It also may be specified in terms of the source of the energy such as incandescent-lamp radiant energy or solar radiant energy. It is preferable to use the term solar radiant energy rather than the commonly used terms sunlight and daylight when dealing with plant physiological problems.

TABLE II

CONVERSION FACTORS FOR ENERGY UNITS

UNITS	ERG	JOULE	GRAM-CALORIE
Erg	1.	10^{-7}	0.239×10^{-7}
Joule	10^{7}	1.	0.239
Gram-calorie	4.18×10^{7}	4.18	1.

The units of radiant energy are the same as those for other forms of energy. Some of the most frequently used units are the erg, joule, and gram-calorie. The quantitative relationship between these units is presented in table II.

The photometric correlate of radiant energy is *luminous energy* or *light* which is radiant energy evaluated with respect to its capacity to produce visual sensation. Light is, therefore, radiant energy spectrally evaluated in accordance with the luminosity curve of the human eye. Radiant energy in the region of 555 mμ, which appears yellow-green to the eye, is more efficient in producing the sensation of brightness than that of any other region of the spectrum. Longer or shorter wavelengths are less efficient. The standard luminosity curve drops to about one-tenth of one per cent. of the maximum value at 410 mμ in the blue-violet and 720 mμ in the red. Radiant energy of wavelengths outside of these limits in the ultraviolet and infrared,

respectively, logically cannot be termed light because it has little or no capacity to produce direct visual sensation. Therefore, such terms as "ultraviolet light" and "infrared light" are misnomers. Within the limits of the visible spectrum, radiant energy may be termed light *only* if it is being evaluated in terms of its capacity to produce visual sensations.

Radiation is the process by which radiant energy is generated and emitted by a source, and propagated through space. Radiation is not synonymous with radiant energy although it frequently has been used in that sense. *Lumination* (*illumination*) is the photometric analog of radiation and is the term applied to the generation and propagation of luminous energy or light.

A *radiator* is any source of radiant energy, regardless of the region of the spectrum involved. The term is usually applied only to that part of the mechanism which actually is radiating, such as the filament of an incandescent lamp or the target of an X-ray tube.

A *luminator* (*lamp, illuminant*) is any artificial source of *luminous energy* (*light*). The term *lamp,* however, is frequently applied to sources of near ultraviolet, visible, and near infrared radiant energy. The term usually includes not only the radiator but the whole permanently attached assembly such as the electrode supports, enclosure, and mounting base.

Special attention is directed to the common but very confusing practice of referring to lamps by trade names. Such terms as Mazda, Osram, Pointolite, Uviarc, Cooper-Hewitt and others are trade names which frequently have little significance to the reader as to the nature of the lamp employed. It is preferable to designate lamps by more descriptive technical terms as incandescent, high pressure mercury arc, sodium arc, or as the case may require. Additional informative data as wattage and color temperature should be included where it is necessary for proper interpretation of the experimental results.

Radiant density is the volume density of radiant energy and expresses the amount of the radiant energy existing within a unit volume of the space under consideration. The unit is the erg per cubic centimeter. *Luminous density* is the analogous photometric term.

Every useful source of energy is evaluated in some unit of power which is a measure of the time rate of flow of the energy. In the case of a source of electrical energy such as a generator, the unit of power is the joule per second or watt. Similarly, sources of radiant energy may be evaluated in terms of the time rate of flow of the radiant energy into the surrounding space which is termed the *radiant flux* of the source. The term radiant flux is not limited to sources alone but is generally applicable to any system involving the transfer of radiant energy. As with any other power term it has nothing to do with the direction of flow of the energy or the dimensional configuration of the system. It may be applied with equal logic to receivers of radiant energy as well as to generators.

Luminous flux is the time rate of flow of luminous energy (light) and is expressed in terms of the *lumen.* The *candle* is the unit of luminous in-

tensity. The unit used in the United States is a specified fraction of the average horizontal candlepower of a group of 45 carbon-filament lamps, preserved at the National Bureau of Standards, when the lamps are operated at specified voltages. This unit is identical, within the limits of uncertainty of measurement, with the international candle established in 1909.

It is evident that the complete surface of a sphere having a theoretically uniform point source of luminous intensity of one candle at its center would intercept a total flux of 4π lumens. Since the area of a sphere is $4\pi r^2$, any portion of the total surface equivalent in area to the square of the radius will intercept a luminous flux of one lumen. The solid angle thus formed, having its apex at the source and its base enclosing a surface on the sphere equivalent in area to the square of the radius, is called a *steradian*. The lumen is therefore a unit of luminous flux and is defined as the luminous flux passing through a unit solid angle, or steradian, from a uniform point source having a luminous intensity of one candle. It is also the flux on a unit surface, all points of which are at unit distances from a uniform point source of one candle.

It frequently is desirable to specify geometrically the radiant flux of the source. Three intensity terms and their photometric analogs have been developed for this purpose. These terms serve to specify the radiant flux emitted by the source as a function of various geometrical units. *Radiant emittance* (*radiancy*) is an expression of the radiant flux emitted per unit area of the source. This term usually is applied to distributed sources of large area such as the fluorescent lamp. The unit is the watt per square centimeter. *Luminous emittance* is the corresponding photometric term.

When sources such as a carbon arc or a projection lamp are employed in optical systems which are large as compared with the dimensions of the radiator, the source may, for all practical purposes, be considered as a point source having no surface area. Therefore, the quantity that is of interest is not the flux emitted per unit area but the radiant flux emitted per unit solid angle, or steradian, which is termed the *radiant intensity*. The unit is the watt per steradian. *Luminous intensity* (*candlepower*) is evaluated in terms of the candle or of the lumen per steradian.

Radiance (*steradiancy*) is a term which is applicable to diffuse sources. It is a combination of the two previous terms and may be defined as the radiant flux per unit solid angle per unit of projected area and is expressed as the watt per steradian per square centimeter. The photometric term is *luminance* (*brightness*) and is expressed in the unit of the candle per square centimeter of projected area, or *stilb*.

The terms *radiant emittance* (*radiancy*), *radiant intensity*, and *radiance* (*steradiancy*) and their photometric analogs are intensity terms which are extensively used in papers in engineering and physics dealing with radiant flux measurements on sources of radiant energy. Their primary use to physiologists is in making calculations concerning the irradiances obtainable from such sources.

Irradiation is the process of interception of radiant energy by an object. It may be considered as the converse of radiation. Thus, it is logical to speak of radiation by a source, in the sense of the process of the emission of radiant energy, and of the irradiation of an object. Irradiation may be specified in terms of the spectral region involved such as visible irradiation, ultraviolet irradiation, or X-irradiation; or in terms of the type of source used, such as incandescent lamp irradiation or solar irradiation. *Illumination* is the corresponding photometric term and is, therefore, the converse of lumination.

The quantitative expression of the degree or magnitude of irradiation is the *irradiance* (*irradiancy*), which is the intensity term dealing with the interception of radiant energy. The irradiance of a surface is the incident radiant flux per unit area. It is very unfortunate that *intensity*, which is a general nonspecific term, has been used so extensively as a substitute for the specific term *irradiance*.

TABLE III

CONVERSION FACTORS FOR IRRADIANCE UNITS

UNITS	ERG/(SEC CM²)	ERG/(SEC MM²)	MICRO-WATT/CM²	GM-CAL/(MIN CM²)
Erg/(sec cm²)	1.	0.01	0.1	1.43×10^{-6}
Erg/(sec mm²)	100.	1.	10.	1.43×10^{-4}
Microwatt/cm²	10.	0.1	1.	1.43×10^{-5}
Gm-cal/(min cm²)	7×10^5	7×10^3	7×10^4	1.

While the defining equations and the basic units for radiant emittance and irradiance as given in table I are the same, it should be noted that the former term applies to sources while the latter applies to interceptors of radiant energy.

Many units of irradiance have been employed in the literature. Some of those units which have been used most frequently are compared in table III. The microwatt per square centimeter is a very convenient and logical unit for biological work. It is the unit which the National Bureau of Standards uses in expressing the irradiances obtainable with their standard lamps.

The photometric analog of irradiance is *illuminance* (*illumination*). It is expressed in such units as the lux, lumen per square meter or metercandle, the phot or lumen per square centimeter, and the footcandle or lumen per square foot. The relationships between these units are given in table IV. The footcandle is an unfortunate unit both as to name and definition because it implies the product of the foot and the candle and because it is not based upon the metric system of units. Since, however, it has attained such general usage in the United States and most of the commercially available illuminometers are calibrated in footcandles, it will undoubtedly continue to be used.

The terms *dark* and *darkness* designate a zero level of the psychophysical entity, luminous energy or light, whose absence is obviously without any

physical influence of any kind. Since there is no convenient radiometric analog, the terms are logically carried over to imply a zero level of radiant energy in the near ultraviolet, visible, and near infrared regions of the spectrum, the limits depending upon the nature of the reactions under consideration. The terms frequently have been used as if they referred to a positive entity capable of a specific physical or chemical influence. Typical examples of such misuse are "the effect of light and darkness," "reactions due to darkness," and "dark reactions." The term "dark reaction" refers to chemical or physical reactions which do not require the direct absorption of radiant energy, but which are associated with the photochemical reactions under consideration. Such "dark reactions" do not require darkness in order to proceed nor are they usually affected by the incidence of radiant energy except indirectly as the precursors or the reaction products may be affected by the photochemical reactions.

TABLE IV

CONVERSION FACTORS FOR ILLUMINANCE UNITS

UNITS	LUX	PHOT	MILLIPHOT	FOOTCANDLE
Lux	1.	0.0001	0.1	0.0929
Phot	10000.	1.	1000.	929.
Milliphot	10.	0.001	1.	0.929
Footcandle	10.76	0.001076	1.076	1.

1 lux = 1 lumen incident per square meter;
= 1 metercandle.
1 phot = 1 lumen incident per square centimeter.
1 footcandle = 1 lumen incident per square foot.

Experimentally it is never possible to exclude all forms of radiant energy. Cosmic rays are always present and all bodies above a temperature of absolute zero are radiating energy which is largely confined to the infrared region of the spectrum. While obviously it is seldom necessary to specify the spectral limits so completely, these considerations should be kept in mind in using terms implying zero levels of radiant energy.

Radiometry is the science of the measurement of radiant energy. The instrument used for such measurements is known as a *radiometer* or a *photometer*. The prefix photo- is used here in the general sense as previously mentioned. *Spectroradiometry* or *spectrophotometry* is the science of the measurement of radiant energy as a function of the wavelength or frequency. The instrument used for the measurement of the spectral energy distribution of the radiant flux is called a *spectroradiometer* or a *spectrophotometer* and consists of a radiometer or photometer in conjunction with a spectrometer. The term *spectrograph* usually is applied to instruments which record the spectrum on a photographic emulsion, but the term has also been extended to those instruments in which the spectrum is recorded mechanically on a chart or electrically recorded with a cathode ray tube. The more logical procedure is to term such instruments *recording spectroradi-*

ometers (**13**) or *recording spectrophotometers* (**2**) in order to distinguish them from photographic instruments.

Spectral terms

The *spectrum* is an optical arrangement of radiant energy with respect to wavelength or frequency. The *spectral* distribution of radiant energy is a function expressing analytically or graphically the relation between the radiant flux and the wavelength or frequency. The relationships between the various wavelength units are given in table V. They are the micron (μ), the millimicron (mμ), the Angstrom (A), and the X unit (XU). The millimicron is the most convenient unit for use in the visible spectrum where only

TABLE V

COMPARISON OF SPECTRAL UNITS

WAVELENGTH			FREQUENCY	WAVE NUMBER
MICRON μ	MILLIMICRON mμ	ANGSTROM A	FRESNEL f	WAVELENGTHS/CM cm^{-1}
0.2	200	2000	1500	50000
0.3	300	3000	1000	33300
0.4	400	4000	750	25000
0.5	500	5000	600	20000
0.6	600	6000	500	16600
0.7	700	7000	428	14300
0.8	800	8000	375	12500
0.9	900	9000	333	11100
1.0	1000	10000	300	10000

0.001 mm = 1 μ = 1000 mμ = 10,000 A = 10,000,000 XU.
The Fresnel, f, is equivalent to 10^{12} vibrations per second.
The wave number is equivalent to the reciprocal of the wavelength in centimeters.
The velocity of radiant energy *in vacuo* is 3.00×10^{10} cm./sec.

three significant figures are necessary. The millimicron sometimes has been designated inconsistently as μμ. Since μ is intended to denote one-millionth, then the unit, μμ, would be one-millionth of a micron instead of one-thousandth of a micron. It should be noted that the English A is now preferred in writing Angstrom; its abbreviation (A) replaces the Swedish Å. The X unit usually is used in the X-ray region of the spectrum.

Frequencies are expressed in units of the vibrations per second and in the fresnel (f). Another unit which is often used in place of frequency is the wave number which is the number of wavelengths per centimeter of path *in vacuo*.

Transmission and related terms

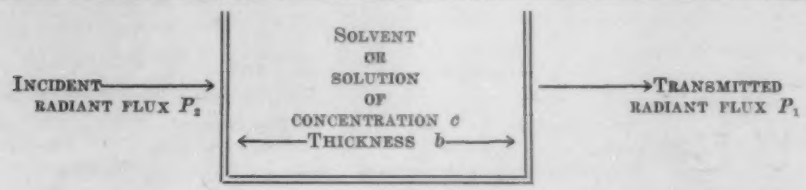
There is less agreement on terms relating to the transmission of radiant energy through matter than there is for the other phases of radiant energy nomenclature. The recommendations presented here are taken from correspondence with the chairman of the Optical Society Committee, the Illuminating Engineering Society report (**5**), and the United States Bureau of Standards Miscellaneous Publication 114 (**1**). Committees of other organi-

zations are now attempting to standardize this entire field of nomenclature and the present recommendations should be considered as tentative, especially in regard to the relationships governing the transmission of radiant energy through substances in solution which are given in table VI.

Transmission is the process by which radiant energy is transmitted through matter. *Diffuse transmission* is that form of transmission in which the transmitted radiant energy is emitted in all directions from the trans-

TABLE VI

RELATIONS GOVERNING THE PROPAGATION OF RADIANT ENERGY THROUGH SUBSTANCES IN SOLUTION



ABSORPTION CELL

$T_{solution} = \frac{P_2}{P_1}$ = *transmittance* (*transmission factor*) of a cell containing the solution

$T_{solvent} = \frac{P_2}{P_1}$ = *transmittance* (*transmission factor*) of the same (or a duplicate cell) containing the solvent

$\mathbf{T} = \frac{T_{solution}}{T_{solvent}}$ = *transmittancy*

$\mathbf{t} = \sqrt[bc]{\mathbf{T}}$ = *specific transmissivity* (BEER's Law)

b = thickness of the solution

c = concentration of the solution

$\mathbf{k} = -\log_{10}\mathbf{t} = -1/bc \log_{10}\mathbf{T}$ = *specific absorptivity*

$\mathbf{i} = -\log_e \mathbf{t} = -1/bc \log_e \mathbf{T}$ = *specific absorptive exponent*

mitting body. *Regular transmission* is that in which the direction of the transmitted pencil of radiant energy has a definite geometrical relation to the corresponding incident pencil.

Absorption is the process by which radiant energy is converted within a body into other forms of energy such as heat.

The *transmittance* (*transmission factor*) of a homogeneous body is the ratio of the radiant flux transmitted by the body to the incident flux. The transmittance takes into account not only the radiant energy which is absorbed directly by the body but also that which is reflected at the surfaces. Therefore it is the term which is used when no correction for surface reflection is made.

The *transparence* (*transmittance*) is the ratio of the radiant flux incident to the second surface to that which enters the first surface. The use of this term takes into account only that radiant energy which is directly absorbed by the body itself and therefore is the transmittance corrected for surface

reflections. When the regular transmission of a non-diffusing body such as a glass filter is measured in air by obtaining the ratio of the transmitted flux to the incident flux, the quantity that is obtained is the transmittance. The transparence of the same body can be obtained by making the measurement of incident and transmitted fluxes with the body immersed in a liquid having the same index of refraction as the body itself in order that surface reflections may be eliminated or at least reduced to negligible values.

Absorptance is the ratio of the radiant energy which is absorbed by a body to that which enters it. It is obtained by subtracting the transparence from unity.

The *transmissivity* is concerned with solids and liquids of constant composition and is the transparence per unit thickness of the substance.

When dealing with substance in solution, it is desirable to correct for solvent absorption as well as reflection losses. *Transmittancy* involves corrections for both of these losses and is applicable only to substances in solution. It is usually obtained from the ratio of the transparence of an absorption cell containing the solution to that of an identical cell containing the solvent. The transmissivity of such a solution is termed the *specific transmissivity* and is the transparence per unit thickness and concentration. The adjective *specific* is added to terms involving substances in solution in order to distinguish them from analogous terms for homogeneous media in which concentration and solvent absorption are not factors. The equation for specific transmissivity is an expression of BEER's Law which states that for solutions of constant thickness, the radiant flux transmitted by a substance in solution is an exponential function of concentration. This law holds for dilute solutions but is frequently only an approximation for concentrated solutions.

The *specific absorptivity* is the common or decadic logarithm of the reciprocal of the specific transmissivity. This quantity has been designated by various terms as specific absorptive index (1), extinction coefficient, etc. The natural or Naperian logarithm of the reciprocal of the transmissivity is the *specific absorptive exponent*.

Reflection is the process by which the direction of the propagation of radiant energy incident *at* a surface is changed. In the case of *regular* or *specular* reflection, the angle of reflection is equal to the angle of incidence. In *diffuse reflection*, the radiant energy is reflected through all angles included within π radians or 180° for each small increment of surface. The scattering of radiant energy from non-metallic films, such as surfaces of white paint pigments, usually involves both reflection and refraction, but the total process is spoken of as reflection and reflection terms are used. *Reflectance* (*reflection factor*) is the ratio of reflected radiant energy to the incident radiant energy.

Refraction is the process by which the direction of propagation of radiant energy is changed in its passage *through* a surface boundary between two media. The *refractive index* is the ratio of the sine of the angle of incidence to the sine of the angle of refraction.

Diffraction is the bending of waves around obstacles, the degree of bending increasing with the wavelength.

When radiant energy of one wavelength is absorbed by a substance and re-radiated at a longer wavelength, the phenomenon is termed *fluorescence*. If the re-emission of radiant energy persists for an appreciable length of time after the removal of the exciting energy, the term *phosphorescence* is usually applied to the phenomenon.

Miscellaneous terms

Polarized radiant energy, commonly called polarized light, is radiant energy in which the vibrations of the electromagnetic waves are confined to a single plane. In the case of non-polarized radiant energy, the vibrations are equally distributed in all planes about the axis of propagation. The energy emitted by most sources is non-polarized.

A *blackbody* is an ideal temperature radiator whose radiant emittance in all parts of the spectrum is the maximum obtainable from any temperature radiator at the same temperature. Such a radiator is called a blackbody because it will absorb all of the radiant energy which is incident to it. A *graybody* is a temperature radiator in which the radiant emittance is less than that of a blackbody at the same temperature. The ratio of radiant emittance of the graybody to that of a blackbody is the same at all wavelengths. This ratio is the *total emissivity* of the graybody and is always less than unity.

The *color temperature* of a source of radiant energy is that temperature at which a blackbody must be operated to give a color match with the source in question. For a source such as an incandescent tungsten filament, the spectral energy distribution may be calculated with considerable accuracy from published data on the spectral energy distribution of a blackbody having the same temperature as the color temperature of the lamp filament. Color temperatures are usually assignable only for sources which have a spectral energy distribution which is not greatly different from that of a blackbody within the visible spectrum.

The *quantum theory* postulates that the exchange of energy from one system to another is discontinuous in nature and that the energy is absorbed or emitted in small units known as *quanta*. The *quantum*, which also is called the *photon*, is therefore the smallest unit of energy which can be absorbed or emitted by an atom or a molecule. The energy of the quantum or photon is $h\nu$ where h is PLANCK's Constant, which is equal to 6.62×10^{-27} erg · sec, and ν is the frequency in vibration per second. Thus the energy of a quantum is proportional to the frequency and is inversely proportional to the wavelength.

EINSTEIN's principle of photochemical equivalence postulates that in a photochemical reaction the primary process involves the activation of one molecule of a substance by one quantum of radiant energy. The *einstein* is the energy required to activate one gram molecular weight of such a substance. The *quantum yield* or *quantum efficiency* of a photochemical reac-

tion is the number of molecules reacting per quantum of radiant energy absorbed or the ratio of the number of moles reacting to the number of einsteins absorbed.

The Physical Methods Committee is indebted especially to Dr. Loyd A. Jones, Chairman of the Colorimetry Committee of the Optical Society of America, to Dr. E. C. Crittenden, Chairman of the Nomenclature Committee of the Illuminating Engineering Society, to Dr. K. S. Gibson, and to Dr. K. W. Meissner, all of whom have very generously cooperated in reviewing and criticizing the manuscript. The Committee wishes to express its appreciation to those others who also have been very helpful in their criticisms of this report.

Agricultural Experiment Station
Purdue University
West Lafayette, Indiana

LITERATURE CITED

1. Davis, Raymond, and Gibson, K. S. Filters for the reproduction of sunlight and daylight and the determination of color temperature. Bur. Standards Misc. Pub. 114. 1931.
2. Harrison, G. R., and Bently, Edward P. An improved high speed recording spectrophotometer. Jour. Opt. Soc. Amer. **30**: 290–294. 1940.
3. Illuminating Engineering Society Report of Committee on Nomenclature and Standards. Ill. Eng. Soc. Trans. **18**: 47–61. 1922.
4. Illuminating Engineering Society Report of Committee on Nomenclature and Standards. Ill. Eng. Soc. Trans. **28**: 263–279. 1933.
5. Illuminating Engineering Nomenclature and Photometric Standard. Ill. Eng. Soc. Trans. **36**: 815–852. 1941.
6. Illuminating Engineering Nomenclature and Photometric Standards. Ill. Eng. Soc. Report ASAZ 7.1. 1942.
7. Ives, H. E. The units and nomenclature of radiation and illumination. Astrophys. Jour. **45**: 39–49. 1917.
8. Jones, L. A. Colorimetry: Preliminary draft of a report on nomenclature and definitions. Jour. Opt. Soc. Amer. **27**: 207–213. 1937.
9. Priest, I. G. Colorimetry. Jour. Opt. Soc. Amer. **4**: 186. 1920. (Full report not published.)
10. Spectrophotometry: Report of the Optical Society of America Progress Committee for 1922–23. Jour. Opt. Soc. Amer. and Rev. Sci. Inst. **10**: 169–241. 1925.
11. Troland, L. T. Report of Committee on Colorimetry for 1920–21. Jour. Opt. Soc. Amer. **6**: 527–596. 1922.
12. Tweney, C. F., and Hughes, L. E. C. Chambers's technical dictionary. MacMillan. 1940.
13. Zworykin, V. K. An automatic recording spectroradiometer for cathodoluminescent materials. Jour. Opt. Soc. Amer. **29**: 84–91. 1939.

SUGARS IN RELATION TO COLOR AND THIOCYANATE SPRAY IN APPLES[1]

R. H. Leonard and R. B. Dustman

Since the discovery of the effect of sodium thiocyanate spray in increasing the amount of red color in apples (**3, 4**) information has been desired concerning the chemical changes occurring in the sprayed fruit and the relationships of anthocyanins to other constituents. It has long been known that carbohydrates are associated with anthocyanins and related in some manner to their formation; consequently a study of the sugars present constituted the first step in seeking a possible explanation of this relationship.

Since the thiocyanate spray increases color development it is reasonable to suppose there may be other differences in chemical composition between sprayed and unsprayed fruits. The red pigment occurs in small and variable amount and cannot be measured quantitatively. For this reason color differences can be expressed in qualitative terms only, but the sugars can be measured and expressed in quantitative values.

The identity of the red pigment in three varieties of apples (**2, 8**) suggests that it may be the same material in all varieties, *viz.*, idaein or 3-β-galactosidyl-cyanidin. Determination of the differences in the sugar content of various regions of the fruit (**1**) has shown that more sugar is present in the stem end than in the calyx end; and that there is more sugar in the colored side than in the uncolored side. Levulose has been found to be the preponderant sugar in the apple (**5**), but dextrose and sucrose also are present in considerable amounts. These three appear to be the only sugars present in the apple in significant quantities.

Materials and methods

The apples analyzed for this report were grown in the University Horticulture experimental orchard at Morgantown in the summer of 1941. All trees received the customary sprays for insect and disease control (**9**). Superimposed upon this treatment in certain varieties were three sprays of 0.1 per cent. sodium thiocyanate, the characteristic effects of which have already been described (**4**). All varieties were harvested in season and stored at 3° C. until analyzed. Summer varieties were analyzed in the early fall and analyses of the winter varieties were completed before the end of the year except those relating to storage studies.

Sampling

Samples showing both a red and a green side of approximately equal area were selected in the orchard from fruit with and without the thiocyanate spray. These samples were analyzed immediately after the apples

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 303.

were harvested. In taking samples of tissue each individual apple was examined to determine the region of maximum color, halved from stem to calyx, the halves held tightly together and another cut made in the same manner at a rotation of a few degrees. This provided two small sectors one of which represented the region of greatest and the other the region of least color. The sectors were kept as uniform in size as possible and constituted about one-sixteenth to one-thirty-second of the fruit.

In comparisons of fruit involving the thiocyanate spray all fruit including both sprayed and unsprayed was always taken from the same exposure and the same large limb of the tree, thus giving the closest comparison possible. All samples analyzed were composites and usually included 25 to 30 apples of each variety.

Comparisons studied included composition of: (1) red versus green side of partly colored apples; (2) thiocyanate-sprayed versus unsprayed fruit; (3) peels from sprayed versus unsprayed fruit; (4) exterior versus interior tissue of sprayed and unsprayed fruit; (5) sugars by alcoholic extraction versus sugars in expressed juice; and (6) changes occurring in the sprayed and unsprayed fruit during storage.

Analytical procedure

The investigator has a wide choice of methods for sampling, extracting, and determining many of the constituents of plants. Even so he frequently combines features from several different procedures as being suitable for one reason or another. This has been done in the present work with sugars. All apples were cored with a no. 6 cork borer before samples of the tissue were taken. Suitable sectors were combined to form composite samples which were heated to 140° C. in aluminum dishes, ground in a Waring Blendor, extracted with alcohol and the extracts prepared and analyzed by the procedure already described (**6**). Dry matter was determined by drying for four and a half hours in a forced draft oven at 105° C. Titratable acidity was determined by electrometric titration of fresh tissue in aqueous solution with 0.1 N NaOH and pH also was determined electrometrically with the use of the glass electrode. The end point of the titration was taken as the point of inflection of the titration curve and occurred at average pH 8.5.

Results

Red versus green side

In the red versus green side comparisons there were nineteen comparisons including eleven varieties with 10 to 160 apples from each variety, or a total of 436 individual apples. The varieties analyzed were Duchess, Wealthy, Red Duchess, Melba, King David, Winesap, York, Stayman, Jonathan, Rome, and Snow. Certain of the varieties provided samples of both thiocyanate-sprayed and unsprayed fruit. The average percentage composition of fresh tissue in the red and green sides of the eleven varieties

is shown in table I. In the table are given also the standard errors for the means, values for the ratios of red to green, and the odds for the differences observed as calculated from the paired samples (7). The mean ratios and odds shown in tables I, II, and III are calculated from the individually paired samples.

The results presented in table I show that dry matter, dextrose, levulose, and total sugar were higher on the red side of the apple. Sucrose, titratable acidity, and pH apparently were no higher on the red side although the value for pH approaches significance. Judging from the ratios, dextrose shows the greatest relative difference between the two sides.

TABLE I

DRY MATTER, SUGARS, AND ACID CONTENT OF THE RED AND GREEN SIDES OF APPLES

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	RED SIDE	GREEN SIDE	MEAN RATIO OF RED/GREEN	ODDS
	%	%		
Dry matter	14.98 ± 0.50	14.07 ± 0.61	1.08 ± 0.03	86: 1
Dextrose	1.69 ± 0.16	1.40 ± 0.12	1.21 ± 0.04	9999: 1
Levulose	5.99 ± 0.19	5.66 ± 0.17	1.06 ± 0.02	499: 1
Sucrose (as invert)	1.71 ± 0.19	1.65 ± 0.19	1.06 ± 0.03	12: 1
Total sugar	9.39 ± 0.34	8.71 ± 0.32	1.08 ± 0.02	9999: 1
Titratable acidity (as malic)	0.50 ± 0.03	0.50 ± 0.04	1.00 ± 0.02	1: 1
pH	3.47 ± 0.03	3.45 ± 0.03	1.01 ± 0.00	18: 1

SPRAYED VERSUS UNSPRAYED

The effect of the thiocyanate spray is indicated by eight comparisons from six varieties with 18 to 38 apples in each sample (total of 234 sprayed, and 207 unsprayed fruits). The varieties used were Melba, Wealthy, Rome, York, Stayman, and Jonathan. The average composition of the sprayed and unsprayed apples for the six varieties is given in table II, together with ratios and odds as given in table I.

Table II shows that dry matter, sucrose, titratable acidity, and possibly levulose were significantly lower in the sprayed than in the unsprayed fruit. The pH was highest in the sprayed fruit.

PEELS FROM SPRAYED VERSUS UNSPRAYED FRUIT

From a casual examination of sprayed fruits it appears that the metabolic change induced in the tissue by the thiocyanate spray occurs throughout the apple. In an attempt to find out if the spray might exert its influence chiefly on the epidermis of the apple, some sprayed and unsprayed samples were stored for a short period after harvest. These apples were pared on an apple-peeler equipped with a circular knife which gave a uniform peel about 1 mm. thick. The peels were assembled in a sample and

TABLE II

DRY MATTER, SUGARS, AND ACID CONTENT OF SPRAYED AND UNSPRAYED APPLES

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	SPRAYED	NOT SPRAYED	MEAN RATIO OF SPRAYED/NOT SPRAYED	ODDS
	%	%		
Dry matter	14.24 ± 0.57	14.82 ± 0.68	0.95 ± 0.02	150 : 1
Dextrose	1.43 ± 0.15	1.31 ± 0.18	1.11 ± 0.08	10 : 1
Levulose	5.85 ± 0.16	6.00 ± 0.20	0.98 ± 0.01	26 : 1
Sucrose (as invert sugar)	1.79 ± 0.25	2.06 ± 0.27	0.84 ± 0.06	99 : 1
Total sugar	9.05 ± 0.31	9.33 ± 0.42	0.98 ± 0.02	12 : 1
Titratable acidity (as malic)	0.40 ± 0.04	0.46 ± 0.04	0.87 ± 0.03	3689 : 1
pH	3.55 ± 0.05	3.44 ± 0.04	1.04 ± 0.01	999 : 1

treated in the same manner as the fleshy tissue, except that a longer period of grinding was necessary to disintegrate the peelings to a comparable state.

Twelve comparisons with eleven varieties were obtained with 8 to 53 apples in each sample. Peels were obtained from 380 sprayed and from 352 unsprayed fruits of Melba, Wealthy, Stayman, Maiden Blush, Red Duchess, Jonathan, McIntosh, Golden Delicious, Grimes, York, and Rome. The composition of the peels of sprayed and unsprayed fruits is given in table III.

Possibly two features are outstanding in the results shown in table III. One of these is that none of the sugars or dry matter is significantly lower in the peels from the sprayed fruit although the value for sucrose approaches significance. The other is that the titratable acidity is significantly lower in the peels of the fruit sprayed with thiocyanate, and the pH is correspondingly higher.

TABLE III

DRY MATTER, SUGARS, AND ACID CONTENT OF PEELS FROM SPRAYED AND UNSPRAYED FRUIT

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	SPRAYED	NOT SPRAYED	MEAN RATIO OF SPRAYED/NOT SPRAYED	ODDS
	%	%		
Dry matter	20.15 ± 1.30	18.95 ± 1.22	1.07 ± 0.04	16 : 1
Dextrose	1.46 ± 0.14	1.42 ± 0.16	1.05 ± 0.04	4 : 1
Levulose	4.31 ± 0.31	4.37 ± 0.24	0.99 ± 0.03	2 : 1
Sucrose (as invert sugar)	1.76 ± 0.24	2.03 ± 0.29	0.87 ± 0.05	23 : 1
Total sugar	7.50 ± 0.48	7.80 ± 0.48	0.96 ± 0.03	8 : 1
Titratable acidity (as malic)	0.218 ± 0.021	0.291 ± 0.020	0.77 ± 0.06	332 : 1
pH	4.30 ± 0.07	4.12 ± 0.07	1.04 ± 0.01	20,000 : 1

Exterior versus interior tissue

The mechanism by which the spray affects the apple tissue is unknown. The thiocyanate may penetrate the epidermis of the apple or it may penetrate the epidermis of the leaves and be translocated to the fruit. With penetration through the apple skin differences might be expected between the outside and inside layers of tissue. If the translocation system was the chief means of transfer then differences between these two general regions would not necessarily be expected.

An attempt was made to determine whether the spray affected one region more than the other. By means of cork borers of suitable size a set of samples was obtained from Wealthy apples that included thirty apples each of sprayed and unsprayed fruit. A plug extending from the skin to the carpels was removed from exactly opposite sides. The outer third of this plug, measured along the length by inspection, was considered as representative of the shell of tissue at the exterior, and the inner third, not

TABLE IV

Dry matter, sugars, and pH value of exterior and interior tissues of sprayed and unsprayed Wealthy apples

Region	Treatment	pH	Dry matter	Dextrose	Levulose	Sucrose (as invert sugar)	Total sugar
			%	%	%	%	%
Outer	Sprayed	3.39	11.15	1.11	4.81	0.39	6.31
"	Not sprayed	3.38	11.06	1.01	4.87	0.52	6.40
Inner	Sprayed	3.37	11.42	1.22	3.51	0.29	5.02
"	Not sprayed	3.38	11.34	1.11	3.45	0.27	4.83

including the carpel, was considered to be representative of the tissue of the inner region. The composition of the tissue from the two regions is shown in table IV.

A statistical analysis was not obtained but the results in table IV do not show a differential spray effect in the two general regions designated. Numerical differences between sprayed and unsprayed fruit are as great for the inner as for the outer region and the values vary only within the limit of error of the determinations.

A second comparison on possible regional differences was carried out in a somewhat different manner and was arranged to compare the epidermis with the layer of tissue directly beneath it. For this comparison thirty-five sprayed Rome apples and a like number of unsprayed fruits were pared with the circular-knife peeler. The outside peel was removed and the next adjacent layer was removed, thus giving two samples from each apple. The individual layers were combined into surface and sub-surface samples and analyzed with the results shown in table V.

In this trial table V shows that the spray apparently affected the peels in a slightly different manner than it affected the sub-surface. Spraying

TABLE V

DRY MATTER, SUGARS, AND ACID CONTENT OF THE SURFACE LAYER IN ROME APPLES AS COMPARED WITH THE SUB-SURFACE LAYER

REGION	TREATMENT	pH	TITRATABLE ACIDITY (AS MALIC)	DRY MATTER	DEXTROSE	LEVULOSE	SUCROSE (AS INVERT SUGAR)	TOTAL SUGAR
			%	%	%	%	%	%
Surface	Sprayed	4.00	0.183	22.70	1.09	3.58	2.38	7.05
"	Not sprayed	4.00	0.189	21.09	1.23	4.50	2.17	7.90
Sub-surface	Sprayed	3.46	0.258	15.94	0.81	5.91	3.02	9.74
" "	Not sprayed	3.51	0.284	14.90	0.82	6.05	3.16	10.03

lowered titratable acidity more in the sub-surface than in the surface and increased the dry matter in the surface more than in the sub-surface layer. Levulose was lowered in the surface to a greater extent than in the sub-surface.

ALCOHOLIC EXTRACT VERSUS EXPRESSED JUICE

Prior to the present work numerous analyses had been made on fresh juice pressed from apples variously treated. It occurred to us that it might be of interest to compare sugar values obtained by alcoholic extraction with sugar values obtained from expressed juice. Two varieties, Melba and Wealthy, were sampled by the extraction procedure already described and the remnants were combined, run through a food chopper, and pressed immediately in a hand press without preheating. The extracts and the fresh juices were analyzed at the same time by the same analytical procedures with the results shown in table VI. Each value listed represents a composite sample of 24 individual apples for Melba and 30 apples for Wealthy.

The values given in table VI show that the results are not entirely consistent for the two varieties studied. Values for fresh juice are expressed

TABLE VI

SUGAR CONTENT OF APPLES AS DETERMINED BY ALCOHOLIC EXTRACTION AND FROM EXPRESSED JUICE

VARIETY	TREATMENT	PORTION ANALYZED	DEXTROSE	LEVULOSE	SUCROSE (AS INVERT SUGAR)	TOTAL SUGAR
			%	%	%	%
Melba	Sprayed	Extract	0.90	4.75	2.41	8.06
Melba	Sprayed	Juice	0.59	4.59	2.53	7.71
Melba	Not sprayed	Extract	0.82	4.93	2.82	8.57
Melba	Not sprayed	Juice	0.35	4.62	3.11	8.08
Wealthy	Sprayed	Extract	1.17	4.16	0.34	5.67
Wealthy	Sprayed	Juice	1.06	5.12	0.08	6.26
Wealthy	Not sprayed	Extract	1.06	4.16	0.40	5.62
Wealthy	Not sprayed	Juice	0.84	5.21	0.08	6.13

as percentage of juice and might be expected to be slightly higher than those expressed as percentage of total tissue (extract). It might be expected, however, that both extract and juice although differing in total values would nevertheless also show the same relative proportions for the three sugars present. This is not the result obtained. In both varieties dextrose does not appear to have been as completely recovered by pressing as were the other sugars.

Changes in composition during storage

A single series of storage trials involving but one variety was made on the effects of the thiocyanate spray on the composition of the fruit during storage. Large samples of sprayed and unsprayed Rome apples were taken from a single large limb on September 30, divided into six uniform lots of thirty-five apples each (three lots within each treatment) and placed in stor-

TABLE VII

Dry matter, sugars, and acid content of Rome apples during storage

Treatment	Date of analysis	pH	Titratable acidity (as malic)	Dry matter	Dextrose	Levulose	Sucrose (as invert sugar)	Total sugar
			%	%	%	%	%	%
Sprayed	October 2	3.54	0.323	12.75	0.86	5.77	2.09	8.72
Sprayed	Nov. 27	3.87	0.233	13.87	1.26	6.06	1.86	9.18
Sprayed	Feb. 2	3.96	0.221	14.03	1.40	5.92	1.62	8.94
Not sprayed	October 2	3.39	0.412	14.43	0.90	5.96	2.33	9.19
Not sprayed	Nov. 27	3.67	0.327	14.43	1.11	6.16	2.31	9.58
Not sprayed	Feb. 2	3.86	0.289	13.50	1.04	5.76	1.69	8.49

age at 3° C. [illegible] days later the first lots were removed and sampled for analysis and the analyses were repeated on the remaining lots at bimonthly intervals. The results of these analyses are given in table VII.

Table VII shows that spraying caused a lowering of sugars at the beginning of the storage period. Here, also, spraying reduced the titratable acidity and increased the pH value, as reported in table II, and although the acid content fell continuously during the storage period the difference due to spraying was relatively the same throughout.

Discussion

In studying the effect of thiocyanate spray on the sugar content of apples it seemed desirable, since thiocyanate affects the amount of red color formed, to establish first the amount of difference, if any, to be expected for the several sugars between colored and uncolored sides of the same fruit. Analyses soon indicated that the thiocyanate spray did not widen this difference and consequently the data for sprayed and unsprayed fruit are combined in the values reported in table I. Where sufficient data were available (tables I,

II, and III) the significance of the differences was calculated. Cases with odds of 30 to 1 or higher are considered to be significant. Dry matter, dextrose, levulose, and total sugar were measurably higher on the colored side. Differences for sucrose are not significant but, interestingly, differences for dextrose are highly significant although dextrose is present in approximately the same amount as sucrose. Of the seven constituents determined, dextrose showed the greatest relative difference between the two sides.

The data in table II show that dextrose was the only sugar showing a higher value in the sprayed fruit, but the difference is not significant. In general, spraying with thiocyanate seems to have lowered most of the constituents determined. It should be stated that in all red varieties, spraying with thiocyanate increased the amount of red color exhibited by the sprayed fruit so that the sprayed samples always showed more red color than the corresponding unsprayed samples. Dextrose and pH tend to have the same relationship in the sprayed to unsprayed fruit as they did in the red to green side of the apples. The other constituents are contrary to what was anticipated (*i.e.*, are lower in the sprayed fruit) by reason of the higher color in the thiocyanate-sprayed fruit. Because of the increase in color it had been anticipated that a relationship between sprayed and unsprayed fruit would be found similar to that which exists between colored and uncolored sides of the same fruit. In this comparison the greatest sucrose content was present with the greatest acid content.

From table III it is clear that the thiocyanate spray had the same influence in lowering the titratable acidity and increasing the pH of the peels as was shown for the whole fruit in table II. Comparisons of peels with fruit in respect to other constituents show less clear cut agreement. It is of interest to note that dextrose and sucrose were present to about the same extent in both peels and whole fruit whereas levulose was about one and one-half per cent. lower in the peels.

The attempt to determine the effect of the thiocyanate spray on exterior and interior regions by means of plugs cut with cork borers failed to show appreciable differences (table IV). It was realized, however, that the regions represented were rather large and general, and a second effort was confined to a much smaller range of tissue. Table V gives the values obtained on two adjacent layers each about one mm. thick. The surface layer comprised the skin and a small amount of adhering fleshy tissue such as might be removed in ordinary mechanical peeling. The sub-surface layer was fleshy tissue only, adjoining the surface layer. As already mentioned this comparison indicates a differential effect on the layers examined. In an earlier publication (**4**) it was stated that "if leaves only are sprayed and the fruits protected, or if fruits only are sprayed and the leaves protected, a color response will be obtained in the fruit in either case, but one less pronounced than where both fruit and foliage are exposed to the action of the chemical agent." These data are in agreement with that observation to the extent that they do indicate some penetration through the skin and subsequent effect on composition as well as on color.

The differences in sugar composition as determined by alcoholic extraction and by analysis of the expressed juice are of interest. It is at once apparent that the absolute values would probably differ since they represent different products analyzed. As the three sugars are all readily soluble, however, the relative proportions of the sugars might be expected to be similar by both methods. Such an expectation is not borne out by the data. It should be stated that the Melba apples were harvested on August 2 at a full ripe stage and the analyses were begun on August 20, whereas the Wealthy apples were harvested on August 13 at a somewhat earlier stage and analysis started on August 26. Notations at the time of sampling describe the Melba as "soft and mellow" and the Wealthy as "firm, hard and juicy." This difference in stage of maturity may help to explain some of the differences shown in table VI, as for example the much higher sucrose content of Melba. It will be noted, however, that levulose is higher in the extracts for Melba and higher in the juice for Wealthy, whereas sucrose is the exact reverse, being higher in the juice for Melba and higher in the extract for Wealthy. With both varieties dextrose seems to have been more completely recovered by alcohol than by pressing, resulting in a higher proportion of dextrose to total sugar when the sugars were removed by alcoholic extraction. The values should be regarded with caution as more data are needed for definite conclusions, but the point appears worthy of notice and perhaps of further investigation.

The outcome of the storage trials set forth in table VII shows that although the thiocyanate spray resulted in a lowering of sugar content at the time of harvest the sprayed fruit apparently did not use sugars as rapidly, as is evidenced by the fact that after four months in storage the sprayed fruit contained more sugars than the unsprayed. In a previous publication (**4**) it was stated that storage records indicated a superior keeping quality for the sprayed fruit. This observation has been confirmed further and may be partly explained by the present analyses on the basis of reduced sugar losses. Respiration studies of sprayed and unsprayed apples in storage would be helpful in clearing up this point.

Summary

1. A study of sugars and certain other constituents in relation to color and thiocyanate spray in apples has been made to include samples from fifteen varieties as follows: Duchess, Red Duchess, Wealthy, Melba, Winesap, King David, York, Stayman, Jonathan, Snow, Maiden Blush, McIntosh, Golden Delicious, Grimes, and Rome. All varieties except Winesap, King David, and Snow provided samples of both sprayed and unsprayed fruit, the three exceptions mentioned having been without thiocyanate treatment.

2. Red and green sides of selected apples showed differences in composition. Dry matter and sugars were higher on the red side, and dextrose showed the greatest relative difference between the two sides.

3. Spraying with sodium thiocyanate reduced the dry matter, sucrose, titratable acidity and possibly the levulose, and increased the pH value.

4. The thiocyanate spray reduced the titratable acidity and increased the pH value of the peels from sprayed fruit, but did not significantly affect the other constituents of the peels.

5. Evidence was obtained indicating that the thiocyanate spray is effective in part by penetration of the skin of the apple and resulting action upon the layers of tissue immediately beneath the skin. Evidence was also obtained indicating that the relative proportions of dextrose, levulose, and sucrose determined by alcoholic extraction of apple tissue may not be identical with those removed by pressing and determined in the juice.

6. Although spraying with thiocyanate caused a lowering of sugars in Rome at harvest time, after four months in storage the sprayed fruit actually had a higher sugar content than similar fruit not sprayed with thiocyanate.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

1. ARCHBOLD, H. K., and BARTER, A. M. Chemical studies in the physiology of apples. XV. The relation of carbon dioxide output to the loss of sugar and acid in Bramley's seedling apples during storage. Ann. Bot. **48**: 957–966. 1934.
2. DUNCAN, IRA J., and DUSTMAN, R. B. The anthocyanin pigment of the Winesap apple. Jour. Amer. Chem. Soc. **58**: 1511–1514. 1936.
3. DUSTMAN, R. B., and DUNCAN, I. J. Red color in apples. Science n.s. **90**: 233. 1939.
4. ———— and ————. Effect of certain thiocyanate sprays on foliage and fruit in apples. Plant Physiol. **15**: 343–348. 1940.
5. EOFF, J. R. Levulose the preponderant sugar of apple juices. Ind. & Eng. Chem. **9**: 587–588. 1917.
6. LEONARD, R. H. Sugars in relation to red color in apples. Thesis, The West Virginia University, 1942.
7. MILES, S. R. A new table of odds based on student's table of probability. Jour. Amer. Soc. Agron. **26**: 341–346. 1934.
8. SANDO, C. E. Coloring matters of Grimes Golden, Jonathan, and Stayman Winesap apples. Jour. Biol. Chem. **117**: 45–56. 1937.
9. SHERWOOD, E. C. Orchard pest control guide. West Virginia Agr. Ext. Circ. 320 (Rev.). 1940.

INFLUENCE OF UNKNOWN FACTORS ON THE VALIDITY OF MATHEMATICAL CORRELATIONS OF BIOLOGICAL DATA[1]

E. L. Welker and F. L. Wynd

(with three figures)

Introduction

A constantly increasing use has been made of mathematical procedures in evaluating biological data during the past decade. Some of these procedures have been mathematically and biologically sound with a consequent advancement of scientific knowledge. On the other hand, there are many accumulations of biological data which do not lend themselves to usual statistical treatment. Attempts to establish statistical correlations in these instances lead to false conclusions which obscure all efforts to interpret the nature of the phenomena under consideration.

One of the most usual methods of investigating a biological problem is the observation of the effect of changes in some aspect of the environment on some measurable response of the organism. The word, "environment," in the present instance, is used with its broadest possible connotation, as signifying all factors and influences within the organism as well as external to it which can act to influence the response of the organism. The frequent use of graphs in the presentation of the relationships between cause and effect is sufficient evidence to indicate how universal is this approach to scientific problems.

The above sentences, at first hand, appear to state only a truism. Yet it is this approach that leads in many instances to erroneous conclusions. The purpose of the present paper is to call attention, as precisely as possible, to the manner in which these erroneous conclusions may enter into the interpretation of phenomena studied in this way.

It is rarely true that a pair of biological characteristics vary exclusively with each other. The more common situation is that each of the characteristics is influenced by a variety of others. For example, the weight of the grain yield of a wheat plant may increase with the size of the root system, but each of these in turn will be affected by the total leaf area, the nutrients in the soil, and many other factors. This interdependency of characteristics complicates the problem of the measurement of the degree of association between the two characteristics being studied; for example, the extent of the root system and the weight of grain produced.

One common statistical measure used in this situation is the Pearson correlation coefficient. It is customary to subject these coefficients to a significance test in an attempt to determine whether or not they are of sufficient

[1] This study was carried out with funds granted the Graduate School of the University of Illinois by the Cerophyl Laboratories, Inc., Kansas City, Missouri.

magnitude to justify the hypothesis that they did not occur by chance. Such justification is said to exist, or not to exist, on the basis of a probability criterion. It is appropriate to point out that the purpose of such a significance test is the allowance for sampling variation, or error as it is often called. Indeed, if we assume the measuring process in the experiment to be perfect and there results a measure of correlation indicating lack of perfect association, then this lack of perfect association between the characteristics being studied would be attributed to variations in other factors in the biological system. The above statement, of course, assumes that the data satisfies all hypotheses required for the use of PEARSON's coefficient as a measure of correlation. Complete solution of the problem then requires the determination of the factors which affect the relationship in question. Failure to recognize the existence of such factors, and to measure or control their effect, frequently invalidates the use of common statistical procedures.

Effect of heterogeneity on the correlation coefficient

The simplest case in which other factors influence correlation coefficients as described above is that in which heterogeneity is present in the data, a situation difficult to avoid in biological research. The meaning of the term heterogeneity can best be explained by means of an example. In measuring the effect of a particular fertilizer on corn production two varieties of corn are used, one being naturally more productive than the other. The data resulting in this case will be heterogeneous due to the corn varieties. This particular situation can be avoided in this instance, but in a more subtle case, it might not be easily avoided. The importance of this heterogeneity is apparent from the following discussion in which it is shown how ordinary correlation coefficients may fail to measure the relationship between variables.

Let us suppose that variables A and B are each affected by some other factors or sums of factors. If all these other factors be held constant, that is, are controlled, three possibilities exist: (1) A and B are independent and the correlation is zero; (2) A and B are positively correlated; (3) A and B are negatively correlated.

In the first case a sample under control of associated factors would be expected to yield approximately zero correlation. In this case a graphical presentation of the data will show points grouped in a circular cluster if the variables are each measured in terms of one standard deviation as unit. Suppose that a second sample is taken with these other factors still controlled but at a level different from that of the first sample. Since A and B may be different in the two samples, four possibilities arise, the nature of which will be evident from figure 1. Each group of data exhibits zero correlation if it is collected under conditions which prevent a differential effect of other factors. For example, if A and B are characteristics of an agricultural crop, which are totally unrelated to each other, any number of samples collected under identical conditions yield approximately zero correlation

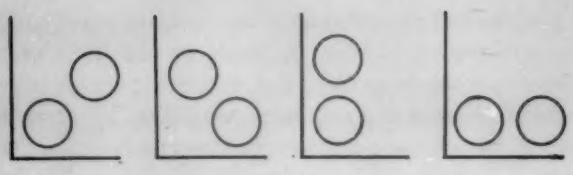


FIG. 1.

between *A* and *B*, chance variation from zero resulting from the sampling. If data obtained for two crops, however, or two harvests from the same planting, two separate fields, or under any other different circumstances are pooled, then spurious correlation is obtained. If unknown factors increase the means of both *A* and *B*, then the pooled data yield a positive correlation coefficient, indicating falsely that *A* varied because of some inherent relation with *B* if the correlation is interpreted in the usual way. If the mean of *A* is lessened, and that of *B* increased, then a negative correlation is obtained. If *A* or *B* simply remain unchanged, then zero correlation results.

In the second instance, suppose that any given group of data collected under identical conditions yields a positive correlation coefficient under all conditions indicating that *A* was inherently related to *B*. If two sets of

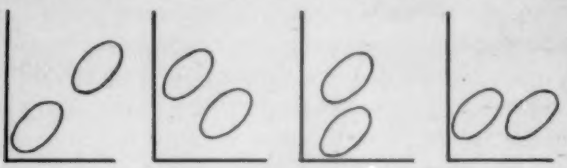


FIG. 2.

such data are pooled, false correlations are again possible, as is evident from figure 2. If unknown factors increase the means of both *A* and *B*, then the correlation of the pooled data will remain positive, but will exhibit an invalid increase in magnitude. It is equally evident that the pooled data may also yield a negative correlation.

In the third instance, groups of data, which are themselves always negatively correlated, may likewise yield spurious correlation coefficients when these groups are pooled. These possibilities are illustrated in figure 3.

The above illustrations show that it is difficult to measure by direct correlation analysis any inherent relation between *A* and *B*, even though such a relation may actually exist. Even though a sample yielded a positive correlation between *A* and *B* one still could not be certain that an increase in *A*

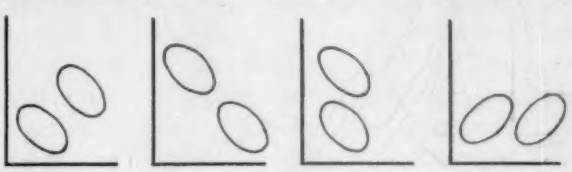


FIG. 3.

would be associated with an increase in *B*, other factors being held fixed. The correlation showing in the sample might be partly or completely the result of variation in other factors.

The only possible mathematical treatment would necessitate quantitative measurements of all factors which affect *A* and *B* in such a way as to permit partial and multiple correlation analysis. Unfortunately, in many biological problems, these various factors cannot even be well defined, and certainly are not measurable in any satisfactory quantitative manner. It should be pointed out that analysis of covariances measures significances of differences between independent estimates of the same covariances, but does not attempt to handle the problem discussed in this paper. It is, of course, possible to combine correlation coefficients of sets of data from the same population by a well known formula. Unfortunately variations in unknown factors implies the possibility of actually having more than one population.

Most variables of biological interest are affected by many factors, both known and unknown. Even when some of these factors are known, it may be impossible to control them or to measure them with any adequate degree of accuracy. For example, suppose that the effect of fertilizer treatments on the relationships between three variable aspects of an agricultural crop are being investigated. These variables could be any measurable response of the plant such as yield of dry matter, percentage of protein, and vitamin C content. The problem becomes one of determining what relationships exist between any two of these variables and how these are affected by the fertilizer treatment.

One may reasonably assume that other factors besides fertilizer will affect the variables *A*, *B*, and *C*. Small and localized differences in the soil, the slope of the field with its consequent effect on irrigation water, undetermined variations in climate, and many other factors certainly exert their respective influences on the physiology of the plant. Randomized arrangements of the plots, or selection of samples, do not eliminate this heterogeneity in the sum total of factors acting on the variables being studied. Neither do large samples, nor large numbers of samples avoid the disturbing effects of the heterogeneity of the environment. It follows that the smaller the area over which the plots are distributed, the more restricted is the time element during growth and sampling; and finally, strangely enough, the smaller the sample itself, the more likely will the sample represent a response conditioned specifically by the fertilizer treatments. It will be seen from the discussion below that this is not merely theoretically true, but is a demonstrable fact which may completely eliminate the possibility of treating any of the data by usual statistical methods.

The difficulty presented by the heterogeneity of the environment is inherent to the nature of biological problems and only under very special circumstances can it be avoided. There is an additional difficulty which further complicates efforts to analyze the data mathematically. Correlations between the variables may be numerically small, and a small number of data

may make it impossible to prove the significance of the observed value. The importance of establishing the magnitude of a correlation may be overstressed by biologists. The nature of the biological problem frequently precludes the collection of a sufficiently large number of data to estimate accurately the magnitude of a correlation and for this reason, the observed value may be discarded as being insignificant; in reality, the correlation actually may be of the greatest importance. The fact that the magnitude of the individual correlation cannot be proved mathematically does not prove that it is therefore insignificant. Biologists frequently appear to be unaware that mathematical tests of significance give only probabilities of the existence of relationships but do not prove independence of the variables when the observed correlation is of insufficient size to justify the assumption of definite relationship on the basis of the given data.

Consistency of the algebraic sign as a test of correlation

In many types of biological research it is of value to determine the algebraic sign of relationships, even though the magnitude of the numerical value is not susceptible to direct proof. For example, if a calculation were based on 15 pairs of data, then a value of 0.64 would be sufficient to establish the existence of positive correlation with only one chance in 100 that a result at least this large would be obtained by pure chance. However, a set of 10 pairs of data between which there was a true correlation would require a value of 0.76 to prove its existence with the same degree of certainty. It thus appears that if a low correlation does exist, its presence may not be determined in a small group of data with a high degree of certainty because the significance test requires a large value which is itself unlikely in this case since the correlation was assumed to be low.

Even though the significance of any one correlation will remain uncertain, it is important to remember that knowledge of the existence of a correlation of one magnitude is just as valuable as that of any other magnitude in an effort to interpret the results. Even though the size of a correlation is valuable information, the problem of proving the existence of the correlation, in the probability sense, irrespective of its numerical magnitude, is likewise very frequently important. In other words, can it be determined that A and B are positively or negatively related, or that no relationship exists?

The following example illustrates the method by which such a decision may be reached. Let us assume that in a given group of data there is zero correlation, and that 10 pairs of data, drawn at random from this group, are studied by the usual methods of statistics. Suppose 10 successive similar samples of data have been drawn and their correlations computed and x are observed to be positive and $10-x$ are negative. What is the probability of this result occurring by chance under the above hypothesis of zero correlation for $x=0, 1, 2, \ldots, 10$? This problem may be translated into terms of drawing balls from a jar which contains an equal number of white and of

black balls. Ten balls are withdrawn at random in succession, and each replaced after it is withdrawn. What is the probability that each of the possible number of white balls will be withdrawn in any group of ten withdrawals? Table I presents these probabilities.

The above probabilities that exactly the indicated number of white balls will be withdrawn are the terms of the expansion of the binomial expression, $(\frac{1}{2}+\frac{1}{2})^{10}$, and the probabilities that at least the indicated number will be withdrawn are the appropriate sums of these same terms.

On the basis of these probabilities, it is possible to determine whether any observed set of positive or negative correlation coefficients in any group of 10 different correlations is likely to occur by chance. For example, if 9 signs are positive, then we would expect this result to occur only once in a hundred

TABLE I

PROBABILITIES THAT CERTAIN NUMBERS OF WHITE BALLS WILL BE WITHDRAWN FROM A HALF-AND-HALF MIXTURE OF WHITE AND BLACK BALLS

NUMBER OF WHITE BALLS WITHDRAWN IN 10 TRYS	PROBABILITY OF EXACTLY THIS NUMBER	PROBABILITY OF AT LEAST THIS NUMBER
10	1/1024	1/1024
9	10/1024	11/1024
8	45/1024	56/1024
7	120/1024	176/1024
6	210/1024	386/1024
5	252/1024	638/1024
4	210/1024	848/1024
3	120/1024	968/1024
2	45/1024	1013/1024
1	10/1024	1023/1024
0	1/1024	1

times in sampling from a population of an actual zero correlation. If such a series should occur in the study of an accumulation of scientific data, we could state that there is positive correlation, and this statement would be true, in the long run, 99 times out of 100 such experiments. The numerical magnitude of this correlation, of course, would remain unknown. The larger the number of individual correlations, the more conclusive will be the results for any given number of signs. It will be seen that we have applied a test for the consistency of the sign as an indication of the probability of the existence of a correlation bearing the same sign by means of refutation of a null hypothesis as is done in other cases. The null hypothesis, that zero correlation actually exists, is refuted if the probability associated with the observed number of positive signs in the samples is small. The hypothesis is not refuted if the above probability is large. The definitions of large and small depend on the significance level which is chosen.

Investigation of heterogeneous biological data

Even though direct statistical correlations cannot yield the desired information concerning the inherent relationship between two variables, certain

facts about this relation can be determined by combining a study of the means of each variable under various conditions, the correlation coefficients of small groups of data, and the consistency of the algebraic sign of these coefficients.

In the study of the relationship between variables of the type discussed above, the investigator may be interested in two aspects of a problem. For example, the effect of fertilizer treatments, and also the effect of successive cuttings on the correlations between the responses *A* and *B*, *A* and *C*, or *B* and *C*. Thus data may be collected at a given time when presumably all factors except fertilizer treatment have been influencing equally all experimental plots, or data may be collected from successive harvests from the plots. In the first instance, all factors except fertilizer treatments are assumed to be constant; in the latter instance, the sum total of all other factors such as changes in climate, soil, and physiological state of the plant, in

TABLE II

CHANGES IN THE MEANS OF VARIABLES FOR HARVEST 1 TO HARVEST 2

	DIRECTION OF CHANGE OF THE MEAN	N	C	Y
For 18 correlation groups, 6 by recombination of data	Increase	0	1	18
	Decrease	18	17	0
For 12 correlation groups, each on separate data	Increase	0	1	12
	Decrease	12	11	0

addition to the fertilizer, is free to operate on the characteristics *A*, *B*, and *C* of the crop.

An investigation similar to the above has been carried out by the writers. The details of the mathematical procedures involved are described below. The botanical features of the investigation will be presented in a future paper.

Measurement of three variables, *N*, *C*, and *Y* were made on each of two successive harvests from a series of plots receiving various fertilizer treatments and from a similar number of control plots. Three possible correlations were computed: r_{NC}, r_{NY}, and r_{CY}. During the course of the statistical analysis it was logical to make certain combinations of data as follows: for each harvest 12 correlations of each of the combinations of variables were obtained. By the combination of certain treatments, 6 additional correlations were calculated, giving 18 values of r_{NC}, r_{NY}, and r_{CY} for each cutting.

During the interval between the harvests, many unidentified factors were operating to change individually each of the 3 variables. These changes in the means of *N*, *C*, and *Y* are tabulated in table II.

It is clear that the means of *N* and *C* were less and that of *Y* was greater for the second harvest. Hence, unknown factors are decreasing *N* and *C* and are increasing *Y* over the period of time elapsing between the harvests.

These variations need not always persist in these directions for every lapse of time between the collection of field samples. Data to be published later show specifically that the changes in C do not always occur in the same direction with a lapse of time.

Now, if our interest is primarily concerned with relationships or correlations between N and C, N and Y, and C and Y it is clear that we must consider correlations and not means. This can be done best by considering all unknown factors affecting the means as a single new variable, X, with sign so chosen that its magnitude may be said to be increasing during the interval between the harvests. It is obvious that the choice of signs is no restriction to our statement that $r_{XN} < O$, $r_{XC} < O$, and $r_{XY} > O$ from the evidence of the means alone, but we can say nothing about the correlations

TABLE III

THE NUMBER OF POSITIVE AND NEGATIVE CORRELATIONS OBTAINED FOR VARIOUS SETS OF DATA AS INDICATED

NUMBER OF CORRELATIONS	HARVEST	r_{NC}		r_{NY}		r_{CY}	
		+	−	+	−	+	−
For 18 correlations, 6 by recombination of data	1st harvest	7	11	15	3	10	8
	2nd harvest	9	9	16	2	9	9
	1st and 2nd harvest pooled	17	1	3	15	2	16
For 12 correlations, each on separate data	1st harvest	5	7	9	3	7	5
	2nd harvest	5	7	10	2	6	6
	1st and 2nd harvest pooled	11	1	2	10	2	10

r_{NC}, r_{NY}, and r_{CY}. We wish to know the answers to the following three questions:

1. For a fixed X, what is the algebraic sign of r_{NC}, r_{NY}, and r_{CY}?
2. For another fixed X, will the correlation r_{NC}, r_{NY}, and r_{CY} have the same algebraic sign as for the first value of X?
3. Is there any method of obtaining information about the relationships, r_{NC}, r_{NY}, and r_{CY} under the hypothesis of allowing variations in X?

In order to answer the first two questions, we shall have to omit from consideration the correlations obtained from the pooled data from the two harvests, since the value of X was different for each harvest. An examination of table III shows that the correlations r_{NC} and r_{CY} are about equally divided as to sign, the divergences from equal numbers of positive and negative signs are so small as obviously to be likely due to chance variation. The correlations, r_{NY}, are predominantly positive in sign. Table IV gives the probability of the occurrence of at least as many positive correlations as were actually observed under the assumption that a zero correlation actually existed.

The probabilities, a to d, in table IV are those associated with the first two cuttings. They can be interpreted as follows. If there had been no

relationship between the variables, then by pure chance one would expect to obtain 15 or more positive correlations in a total of 18, about 4 times in 1000 experiments of this kind, each involving 18 random correlations. We are, therefore, justified in view of this comparatively low probability to assume that a relationship between the variable concerned probably does exist. The definition of a "sufficiently low" probability should depend to a large extent on the judgment of the experimenter. Certain conventional levels of significance are well known. If the correlations based on both harvests are considered, then a very significant result is obtained; namely, only 3 chances in 100,000 that 31 values of a given sign would be obtained in 36 correlations of inherently unrelated data. Such a combination seems justifiable.

There is yet the problem of answering the third question, concerning what can be said about r_{NC}, r_{NY}, and r_{CY} if X be allowed to vary. Since r_{XN} and r_{XC} agree with each other in sign, but disagree in sign with r_{XY} as seems

TABLE IV

PROBABILITY THAT THE OBSERVED NUMBER OF POSITIVE CORRELATIONS WERE OBTAINED BY CHANCE FROM A POPULATION OF A TRUE ZERO CORRELATION

HARVEST	NUMBER OF CORRELATIONS			PROBABILITY THAT AT LEAST THIS NUMBER OF POSITIVE CORRELATIONS WERE DUE TO CHANCE
	POSITIVE	NEGATIVE	TOTAL	
a 1st	15	3	18	0.004
b 2nd	16	2	18	0.0007
c 1st	9	3	12	0.07
d 2nd	10	2	12	0.02
a + *b*	31	5	36	0.00003*
c + *d*	19	5	24	0.003

* A conservative approximation by normal curve tables due to the size of the numbers involved.

likely from a study of the means, one would expect that if X is allowed to vary, r_{NC} would be positive while r_{NY} and r_{CY} would be negative. Table III shows that when data from both harvests are pooled, these results are actually obtained. For instance, the signs of r_{NC} are about equally positive and negative for any single harvest, that is to say, when X is constant; but when the data are pooled, the algebraic signs are 17 positive to only 1 negative. Table IV shows that such a result would be obtained by chance less than 7 times in 10,000 such experiments. It therefore appears reasonable to assume that the unknown factor X is effective in causing the observed positive sign of the correlations of the pooled data, N and C. The signs of r_{NY} are predominantly positive when X is constant, but 15 are negative and only 3 are positive when X is allowed to exert its influence. Table IV shows that this result would occur by chance only 4 times in 1,000 such experiments. Hence the unknown factor may be assumed to be the cause of a negative correlation of the pooled data, N and Y. The signs of r_{XY} are about equally positive and negative when X is constant, but 16 are negative and only 2 are positive when factor X is operating. Table IV shows that this distribution of alge-

braic signs would occur only 7 times in 10,000 such experiments which shows the effect of X in causing negative correlations of the pooled data, C and Y.

Summary

1. Groups of biological data obtained under different conditions cannot be pooled in an effort to increase the significance of statistical correlations by increasing the number of pairs of variates.

2. Direct correlation procedures are inadequate to prove or disprove inherent relationships between some biological characteristics, because factors, usually of unknown nature, may falsely exaggerate or diminish the numerical magnitude of the observed correlation coefficient. These factors may even change the algebraic sign of correlations obtained from the pooled data.

3. Under certain conditions, consistency of sign of a number of correlations may be used to establish relationships, even though the conditions of the experiment may preclude the usual statistical methods of establishing the numerical magnitude of any single correlation coefficient.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

BORDEAUX INJURY TO FOLIAGE AT LOW TEMPERATURES

C. E. YARWOOD

(WITH TWO FIGURES)

That bordeaux spray increases transpiration has been observed by many investigators (**4**). That bordeaux spray decreases leaf temperature has been demonstrated by TILFORD and MAY (**5**). These two facts singly or in combination might lead one to expect that bordeaux spray would increase the susceptibility of foliage to cold injury. The common observation that bordeaux injury to foliage, presumably copper injury, is sometimes greater during cool, wet weather, is probably unrelated to the subject under discussion.

Potatoes showing light late blight (*Phytophthora infestans*) infection were sprayed with bordeaux in two localities about a mile apart near San Juan, California, on September 28, 1941. In one of the localities the plants were severely and suddenly injured by some cause other than late blight, apparently frost, several days after spraying. Potatoes in the other locality were not severely injured. On October 16, the sprayed and unsprayed plants in the unfrosted locality appeared similar in vigor with about 75 per cent. of the leaves still living. At the same time in the presumably frosted locality the unsprayed plants had about 10 per cent. of their leaves living, while the bordeaux-sprayed plants had only about 2 per cent.

Dr. HEUBERGER of the Connecticut Agricultural Experiment Station informs the writer that he and Dr. HORSFALL observed that bordeaux-sprayed cantaloupe foliage was more severely injured by frost than unsprayed foliage in Connecticut in 1940. Also, Dr. RUEHLE of the Florida Agricultural Experiment Station writes that he has observed greater frost injury to bordeaux-sprayed than to adjacent unsprayed potatoes at Homestead, Florida. To the contrary, BUTLER (**1**) writes: "We are told that plants sprayed with bordeaux mixture resist white frost better than non-sprayed plants, which means nothing more, of course, than that sprayed leaves do not cool as rapidly as non-sprayed leaves." The matter of bordeaux injury as related to low temperature was therefore investigated under controlled conditions.

Materials and methods

For all tests 1 per cent. bordeaux was prepared by adding equal volumes of 10 per cent. $CuSO_4 \cdot 5H_2O$ and 10 per cent. CaO to the required amount of water. Plants were sprayed with an atomizer at 35 pounds air pressure and were used for subsequent tests within a few hours after the spray had dried. All tests were arranged in paired comparisons so that one set of leaves was sprayed with bordeaux and a similar set of leaves was left unsprayed. The pairs used for comparison were opposite primary leaves, as in some tests with beans, similar shoots from the same potato tuber, or simi-

lar plants. All tests were replicated and repeated and were performed in the greenhouse, laboratory and cold rooms at Berkeley during the months of November, 1941, to December, 1942.

To measure transpiration, detached bean plants with their stems in cotton-stoppered flasks were placed in the dark test environment and weighed at intervals. After completion of the test the leaf area was measured and the water loss was calculated on a unit area and time basis. Leaf area was taken as the total plane surface area and is thus twice the measured planimeter area. It is realized that the effect of bordeaux in increasing transpiration is sometimes greater for detached leaves than for potted plants (**4**), but this does not appear important for purposes of this study.

TABLE I

EFFECT OF BORDEAUX SPRAY ON LEAF TEMPERATURE AND LEAF INJURY AS RELATED TO LOW TEMPERATURE

TEST SURFACE	DEPRESSION OF TEMPERATURE; AMOUNT BY WHICH TEST SURFACE WAS LOWER THAN AIR TEMPERATURE			INJURY OF PLANTS HELD AT 0° C. FOR 4–12 HOURS AND THEN RETURNED TO GREENHOUSE
	DIFFUSE DAYLIGHT OF LABORATORY AT 21°, USUALLY ABOUT 50% R.H.	7° C. DARK ROOM 64% R.H.	0° C. DARK ROOM 70% R.H.	
	°C.	°C.	°C.	*rating**
Wet thermometer bulb	−6.1	−2.5	−1.4	
Young bean, unsprayed	−2.3		−0.3	
" " + bordeaux	−3.5		−0.6	
Medium bean, unsprayed	−2.0	−0.2	−0.2	0.03†
" " + bordeaux	−2.6	−0.9	−0.3	3.3†
Rusted bean, unsprayed	−1.5	−0.5		
" " + bordeaux	−2.2	−0.5		
Mildewed bean, unsprayed	−1.5	−0.1		
" " + bordeaux	−2.0	−0.5		
Potato, unsprayed	−2.3			1.7‡
" + bordeaux	−3.3			3.5‡

* Scale of 0 to 10 in which 0 indicates no apparent injury and 10 indicates leaves are dead.

† Each value is the arithmetic average of ratings of 7 plants.

‡ Each value is the arithmetic average of ratings of 14 shoots.

Temperature was measured to nearest 0.1° C. with glass thermometers with large cylindrical mercury reservoirs. The thermometers were graduated to 0.2° C., and each was calibrated before use. To measure leaf temperatures the test leaf attached to the potted plant was folded once around the mercury reservoir and held tightly in place with a clip. In this way about 300 sq. mm. of the total of 366 sq. mm. outside area of the mercury reservoir was covered with and in tight contact with one thickness of leaf and the remainder, 66 sq. mm. was exposed to the environment. One test was arranged so that the entire mercury reservoir was in contact with one layer of leaf and no difference in temperature from that obtained by the above

method was detected. The temperature of bordeaux-sprayed leaves was measured with the spray on the outer leaf surface with respect to the thermometer, though the leaf surface in contact with the thermometer was also covered with a bordeaux deposit in some tests. The air temperature was always followed simultaneously with a similar thermometer. The use of mercury thermometers for the measurement of leaf temperatures is generally not regarded with favor by plant physiologists but the writer believes that the method gave reasonably reliable measurements in these tests. In simultaneous temperature measurements of 8 bean leaves by this method, and with the average leaf temperature 2.7° C. below air temperature, the maximum difference between opposite leaves was 0.2° C.

Relative humidity of the environment under study was measured with a Friez HA–2 hand-aspirated psychrometer. The wet bulb depression as given in table I, however, was measured with thermometers of the same type used to measure leaf temperature, with the mercury reservoir covered with a thin linen jacket and dipped in distilled water before use. This thermometer with the wet bulb was held stationary in the same environment as test plants during leaf temperature measurements and wet bulb depression as taken from this thermometer was directly comparable with the observed leaf temperatures.

Cold injury was brought about by exposing potted test plants in dark rooms held at approximately 0° C., 2° C., or 7° C., and where the air was kept continuously circulating by means of fans. Injury to bordeaux-sprayed leaves was usually apparent in 4 hours as a wilting of the sprayed foliage. After 2 to 16 hours the plants were returned to the greenhouse where the injured foliage usually did not recover. About 24 hours after the plants had been returned to the greenhouse, injury was rated on an arbitrary scale of 0 to 10, in which 0 indicated no apparent injury, and 10 that all the foliage was killed. A rating of 3 would then indicate that 30 per cent. of the leaf area was killed. Ratings of individual plants were made in unit figures, but averages of several ratings are expressed as decimal figures. In the quantitative measurements of cold injury to cantaloupes, the methods are presented with the results.

Results

Effect of bordeaux on transpiration

Bordeaux spray greatly increased the transpiration of greenhouse-grown beans. The ratio of water loss per unit area for bordeaux-sprayed, to the water loss from unsprayed foliage, is termed the bordeaux ratio. In one temperature series the water loss in grams per square decimeter per hour for the unsprayed, and the bordeaux ratio was as follows: 0.29 grams and 2.3 at 7° C.; 0.36 grams and 2.6 at 13° C.; 0.5 grams and 6.0 at 22° C.; 0.89 grams and 3.7 at 31° C.; and 1.7 grams and 2.9 at 37° C. Results at 0° C. were more difficult to secure because the water loss was slower and because bordeaux-sprayed bean plants usually wilted in a few hours; but in one test the

values were 0.11 of a gram loss and a ratio of 1.2, and in another 0.10 gram loss and a ratio of 1.5. The rate of water loss usually decreased rapidly with time at 0° C. Since these temperature comparisons were not performed under similar conditions of relative humidity and air circulation they should not be emphasized; but they do indicate that bordeaux spray increases transpiration over a wide temperature range, and even at 0° C. From the results secured it appears that the bordeaux ratio is less at high or low temperatures than at intermediate temperatures, and is always significantly greater than 1.

Effect of bordeaux spray on leaf temperatures

Bordeaux spray consistently reduced bean and potato leaf temperatures and the amount of reduction varied with the experimental conditions. A total of 948 records of leaf temperature and 316 of the corresponding air temperature were made. Some representative values are given in table I and some in the following discussion.

Temperature depression due to bordeaux was less at low than at moderate temperatures. This is indicated by the depression values of 2.6°, 0.9° and 0.3° C., for bordeaux-sprayed beans at 21°, 7°, and 0° C., respectively (table I). Because temperatures were read to 0.1° C. the relative error of temperature depression values was greater at low temperatures. The smaller temperature depression at low temperatures is to be expected from the lower actual transpiration and lower bordeaux ratios at low temperatures.

Young bean leaves with or without bordeaux spray showed more temperature depression than older leaves. This is indicated by the depression values of 3.5°, 2.6°, and 1.1° C. for young, medium, and old, bordeaux-sprayed bean leaves, respectively (table I).

When the lower surface of a bean leaf wrapped around a thermometer bulb was the transpiring surface exposed to atmospheric conditions, the temperature depression was lower than when the upper leaf surface was exposed. In one test the temperature of bordeaux-sprayed bean leaves was 2.7° C. below air temperature when the upper leaf surface was exposed to the atmosphere, and 3.6° C. below air temperature when the lower surface was exposed. This is presumably owing to the greater transpiration and resultant cooling of the lower leaf surface. Although the exposed surface of the leaf was the principal factor determining temperature depression, bordeaux treatment of the unexposed leaf surface against the mercury bulb of the thermometer also increased temperature depression somewhat, though no good reason for this is known. The data in table I were secured with the lower leaf surfaces exposed to the atmosphere.

The temperature depression of leaves in an air current of 450 feet per minute (about 5 miles per hour) was less than that of leaves in the relatively still air of the laboratory. In one representative test at 20° C. and 50 per cent. relative humidity, the temperature depressions for bordeaux-sprayed young, medium, and old bean leaves with the lower surface exposed to the environment were 2.8°, 1.7°, and 0.2° C., respectively, with an electric fan

turned on, and 3.5°, 2.3°, and 0.4° without the fan. Other types of leaf surfaces gave similar differences. In the same test as the above the temperature depression of the wet bulb was 6.9° with the fan turned on and 5.7° without the fan.

In another test of this type a bean plant was attached to a photometer and with one leaf wrapped around a thermometer. The plant was held for alternating 10-minute periods in still air, and in air at 600 feet per minute, for a total of 90 minutes. Each time the fan was turned on the leaf temperature rose (as much as 1.3° C. in 2 minutes) and each time the fan was turned off the leaf temperature fell, but no corresponding change could be detected in the rate of transpiration. These results are apparently the opposite of those of CURTIS (**2**) who states ". . . fanning may cause a drop in leaf temperature of from a few to 10° C. or more . . ." and ". . . transpiration is often reduced when the shoot is vigorously fanned." The explanation is probably that CURTIS's observations were made in strong artificial light while those of the writer were made in diffuse winter daylight in the laboratory. In the observations of CURTIS leaf temperatures were presumably above air temperature, while in this writer's tests of the type recorded here, leaf temperatures were always below air temperature.

MILLER (**4**) has pointed out that most records of transpiration increase due to bordeaux were with greenhouse-grown plants. The writer believes that the temperature depressions recorded above are due primarily to transpiration and it might therefore be expected that temperature depression would be greater for bordeaux-sprayed plants grown in the greenhouse than for those grown outdoors. All records of bean leaf temperature reported here are for greenhouse-grown plants but a comparison was made of potatoes grown outdoors with those grown in the greenhouse. In one test in the greenhouse on February 24, 1942, the temperature depression for control and bordeaux-sprayed leaves was 2.9°, and 3.4°, respectively, for potatoes grown in the greenhouse and 1.9° and 3.1° for potatoes grown outdoors. This record indicates a temperature depression due to bordeaux for plants grown outdoors, as well as for greenhouse plants.

Bordeaux also increased the temperature depression of bean leaves infected with powdery mildew, *Erysiphe polygoni*, D.C., and of leaves infected with rust, *Uromyces phaseoli* (Pers.) Wint. (table I).

EFFECT OF BORDEAUX AND LOW TEMPERATURE ON LEAF INJURY

Bordeaux-sprayed and control bean, potato, tomato, cucumber, cantaloupe, and cabbage plants were exposed in the dark to 0° C. and about 70 per cent. relative humidity for 2 to 20 hours. Except for cabbage, injury resulted when bordeaux-sprayed plants were exposed at 0° C. for 12 hours or more. The controls were also injured in some tests but in every case (total of 14 tests) the bordeaux-sprayed plants were more severely injured than the unsprayed. Cold injury was usually apparent as wilting while the plants were in the cold rooms. Wilted, unsprayed plants frequently recov-

ered when returned to the greenhouse but wilted sprayed leaves usually died. Control plants with similarly sprayed leaves left in the greenhouse continuously showed no apparent injury.

Average data for bean and potato, which were fairly sensitive to bordeaux injury at low temperatures, are given in table I and potato shoots from one test are illustrated in figure 1. That the injury was associated with water loss from the foliage and was not a direct injury due to bordeaux at the low temperatures tested is strongly indicated by the fact that bordeaux-sprayed and unsprayed beans at 0° C., but in moist chambers where transpiration was reduced or nil, showed no injury in 2 tests.

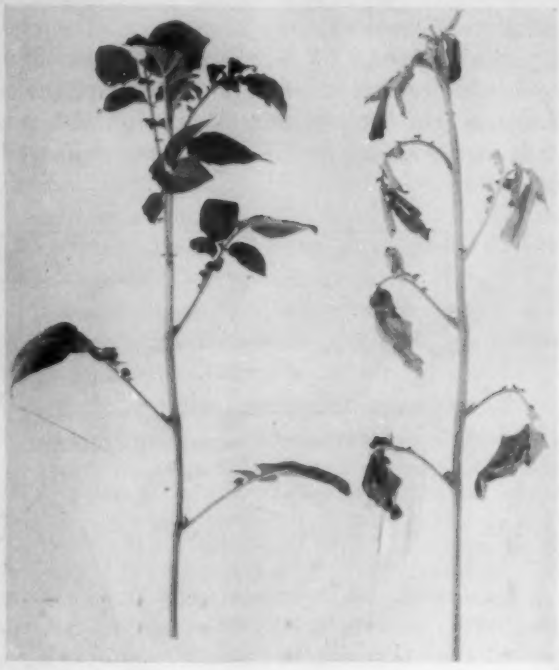


FIG. 1. Effect of bordeaux spray on cold injury to potatoes. Shoots were on the same plant. Shoot on right was sprayed 5 P.M., September 5, with 1 per cent. bordeaux. Shoot on left was unsprayed. Both were exposed to 0° C. in the dark from 7:30 P.M., September 5, to 9 A.M., September 6, and then returned to the greenhouse. Detached and photographed 9 A.M., September 7.

With greenhouse-grown tomatoes, exposure of bordeaux-sprayed plants to low temperature caused severe injury or killed the younger leaves, but caused no apparent injury to the older leaves in 2 tests.

Of the 6 plant species tested cantaloupe was apparently most sensitive to bordeaux injury at low temperatures. Unsprayed cantaloupes exposed to 0° C. for 16 hours overnight were injured in all tests, and bordeaux-sprayed plants were usually killed by the same exposure. To secure quantitative data the green weight of the leaves of sprayed and unsprayed plants

was measured 10 to 18 days after they had been subjected to a series of test temperatures for one overnight exposure. Average results are presented in figure 2. Exposures to 0°, 2°, and 7° C. greatly depressed the yield of unsprayed plants, and the yield reduction was even greater for the bordeaux-sprayed plants. At 13° to 34° C. temperature alone had no apparent effect on yield, and reduction in yield due to bordeaux was progressively less with increase in temperature. At 40° there was a fairly definite reduction in yield due to high temperature, with no apparent difference between bordeaux and control plants. At 0° to 2° C. the green weight of living leaves from bordeaux-sprayed plants was only 20 per cent. of the green weight of control plants, while at 28° to 40° C. the green weight of the bordeaux-sprayed plants was 98 per cent. of the green weight of the control plants. These results indicate that the bordeaux injury studied here is not merely an aggravation of injury caused by injurious temperature, but occurs only at low temperature.

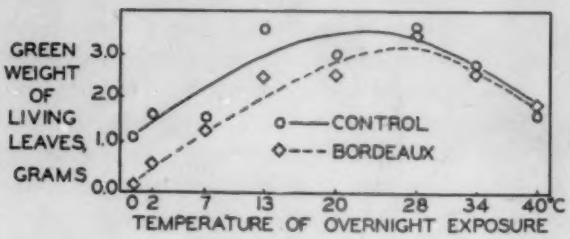


FIG. 2. Effect of bordeaux spray and exposure in the dark to a series of temperatures on the yield of cantaloupe plants. The potted plants were sprayed with bordeaux and allowed to dry, then placed at the test temperature for 16 hours from 4 P.M. to 8 A.M. and returned to the greenhouse. Ten to 16 days later all living leaves were weighed and the above is the average of 4 tests, with one control and one sprayed plant at each temperature in each test. The results clearly show that exposure of plants to low temperatures greatly decreased the weight of plants and that this reduction was greater on bordeaux-sprayed plants.

Cabbage was the only plant tested which did not show bordeaux injury at low temperatures. In one test, control plants and plants sprayed with 1 per cent. bordeaux plus 0.1 per cent. spreader were exposed for 16 hours in the dark to 0°, 2°, 7°, 13°, 20°, 28°, 34°, and 40° C. and no injury due to bordeaux or to low or high temperature was observed on any of the plants.

Discussion

The writer believes that the observed effect of bordeaux mixture in (a) increasing transpiration, (b) decreasing leaf temperature, and (c) increasing leaf injury at low temperature, are partially interdependent and that the two latter effects are partly the result of the preceding. To say that injury occurred at 0° C. as a result of increased transpiration and lowered leaf temperature due to bordeaux spray, is, of course, another way of saying that bordeaux-sprayed plants showed cold injury at higher air temperature than

unsprayed plants. The low temperature injury considered here could logically occur only at relative humidities less than 100 per cent. and would therefore be less likely in a white frost than in a black frost. It has not been determined in this study with potted plants whether the cold injury observed was the result of drying out of the leaf due to decreased root absorption at low temperatures (**3**) or due to other causes. Since the injury occurred at temperatures far above the freezing point, however, it is obvious that the injury is not associated with the formation of ice in the leaf tissues, and cannot be frost injury. Since wilting was an immediate symptom of bordeaux injury at low temperatures, it seems clear that the injury is directly associated with water relations. The writer believes the injury was probably a result of the increased water loss due to bordeaux, associated with decreased absorption and translocation of water at low temperatures.

The importance of radiation in determining leaf temperatures in this study is not known. It is commonly believed that radiation of leaf surfaces to the sky is largely responsible for their marked cooling below air temperature on clear nights. No reported effect of bordeaux deposit on this radiation has come to the writer's attention, though CURTIS's observations [(**2**) fig. 4] indicate that lime deposit on leaves may have increased leaf radiation and decreased leaf temperature. Unfortunately the only controls were the same leaves after the lime had been removed by wiping. This effect of radiation may have been important in determining the difference in injury between bordeaux-sprayed and unsprayed plants in the field, but the writer does not believe that radiation was of importance in the laboratory studies reported here.

Bean, potato, cucumber, and cantaloupe plants, which showed bordeaux injury at low temperatures in these tests, would probably be horticulturally classified as sensitive to cold. Cabbage, the most tolerant to cold under natural conditions of all the plants tested, showed no bordeaux injury associated with low temperature. Though the numbers tested are small, it appears from the results that bordeaux injury associated with cold would be more pronounced on plants naturally sensitive to low temperatures.

Summary

In a field of potatoes apparently injured by frost, bordeaux-sprayed vines were more severely injured than the unsprayed.

In tests with greenhouse plants, bordeaux spray increased water loss from beans at temperatures from 0° to 37° C., decreased leaf temperatures of beans and potatoes by as much as 3.6° C., and caused injury to bean, potato, cucumber, and cantaloupe foliage at 0° C. Unsprayed cantaloupe plants were injured by exposure to 0° to 2° C., but bordeaux-sprayed plants were more severely injured. Control and bordeaux-sprayed cantaloupe plants exposed to 20° to 34° C. showed no marked bordeaux or temperature injury, and plants exposed at 40° C. showed temperature injury without bordeaux injury. Cabbage plants showed no bordeaux or temperature injury at exposures of 0° to 40° C.

It is believed that the increased transpiration and decreased leaf temperature as a result of bordeaux spray are associated with the bordeaux injury to foliage at low temperatures.

DIVISION OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BUTLER, O. Bordeaux mixture II. Stimulatory action. New Hampshire Agr. Exp. Sta. Tech. Bull. 21. 1922.
2. CURTIS, OTIS F. Leaf temperatures and the cooling of leaves by radiation. Plant Physiol. **11**: 343–364. 1936.
3. KRAMER, PAUL J. Species differences with respect to water absorption at low soil temperatures. Amer. Jour. Bot. **29**: 828–832. 1942.
4. MILLER, EDWIN C. Plant physiology. McGraw-Hill Book Company, New York. 1938.
5. TILFORD, PAUL E., and MAY, CURTIS. The effect of bordeaux mixture on the internal temperature of potato leaflets. Phytopath. **19**: 943–949. 1929.

NUTRITIONAL RELATIONSHIPS OF BORON AND INDOLEACETIC ACID IN HEAD LETTUCE

ARTHUR D. MOINAT

(WITH TWO FIGURES)

Lettuce, which is being grown in ever increasing quantities, suffers from a number of nutritional disorders which have been the subject of considerable speculation and study. Much of the difficulty with lettuce is evidently the result of the type of forcing culture which is used to obtain the most firm and succulent heads. Among the micro-nutrient elements, boron has been given considerable attention in recent years since it is now known to be more widely deficient in the soils of the country than was believed earlier. For lettuce it has been shown that boron deficient plants show necrotic tissue near the leaf margins and over the growing point. Such plants have been photographed and described by MCHARGUE and CALFEE (**2**) and others. Some have maintained that the symptoms are the same as those of the so-called tip-burn which has been the most serious disease of head lettuce in many localities. Perhaps that which the grower includes under the designation of tip-burn is in reality more than one physiological disorder. MCHARGUE and CALFEE found that 0.5–1.0 p.p.m. of boron in the culture medium was optimum for the production of lettuce leaf tissue dry matter, although they noted that quantities of boron which were toxic for young plants, might give favorable results with older plants.

The function of boron in the plant is unknown but several hypotheses as to its possible activities have been proposed. MCMURTREY (**3**), in his review of nutritional deficiency symptoms, points out the importance of boron for proper functioning of the growing points. SCHROPP and ARENZ (**5**) found that a deficiency of boron was associated with greater uptake of nitrogen. Severe deficiency symptoms resemble those of ammonia poisoning. These authors also suggested an indirect function of boron, that of preventing swelling of the plasma colloids which might interfere with sap rise in the stem. MINARIK and SHIVE (**4**) found that, lacking an optimum boron supply, there was a subnormal quantity of calcium in the tissues of soybeans. SHIVE (**6**) has also reported a direct relationship between the soluble boron in plants and quantity of soluble calcium in both monocotyledons and dicotyledons. MINARIK and SHIVE found that within the range of boron concentrations used, the percentage of water in the tissues of soybean leaves decreased with increasing concentrations of boron in the nutrient solution.

A possible function of boron was suggested by EATON (**1**) as being a necessary element for the formation of an auxin, such as indoleacetic acid. He was of the opinion that those plants in his tests which were grown with a limited supply of boron showed an increased growth rate when indoleacetic acid was sprayed on the leaves. Similarly SKOOG (**7**) found an inter-rela-

tionship between zinc and indoleacetic acid suggesting the necessity of zinc for auxin synthesis or for the maintenance of auxin in an active state.

In the studies reported herein, a number of concentrations of boron in the nutrient solution for head lettuce formed one group of tests while across the boron series indoleacetic acid in several concentrations was applied to the leaves as a spray. With such a set-up it was hoped that we would be able to study the inter-effect of the two substances and also their relationship to the tip-burn disease.

Materials and methods

Series I

Lettuce of the variety New York was grown in sand cultures using Hoagland's nutrient solution. The basic solution was made up with 0.005 M KNO_3, 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4. Copper chloride was added to the basic solution at the rate of 0.02 p.p.m. Cu; $MnCl_2$ to get 0.4 p.p.m. Mn; $ZnCl_2$ to get 0.047 p.p.m. Zn. Ferric citrate was applied at the rate of 1 p.p.m. with each addition of nutrient solution to the sand. Nutrient solution was applied every second day or more often if necessary to prevent wilting. Each week the sand of each plant container was thoroughly flushed out with distilled water.

Boron was applied as boric acid in ten different concentrations of 0.0, 0.005, 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, and 5.0 p.p.m. of boron to as many groups of plants. Each group consisted of 21 plants, 3 plants to the container, and seven containers.

Indoleacetic acid was mixed with a lanolin-in-water emulsion, using sodium oleate as the emulsifying agent and making the following concentrations of indoleacetic acid: 0.0 (lanolin only), 5, 10, 20, 30, and 40 p.p.m. After the first three weeks of application, the concentrations of indoleacetic acid were increased to 0.0, 100, 150, 200, 250, and 300 p.p.m. The solutions were applied to the leaves and growing points of each plant each day with an atomizer. Of the seven pots of plants for each boron concentration, each pot of plants received a different concentration of indoleacetic acid spray including a distilled water spray for one pot of plants in each group.

On harvesting, the plant material was weighed and then reduced to dry weights in a forced draft oven at 80° C. Sand was separated from roots for dry weight determination by floating off the dried roots after a light grinding in a mortar. The plants were grown in earthenware jars in the greenhouse at the University of Chicago during the summer.

Series II

Plants for this group were grown in the greenhouse during the late autumn. Properly treated cans were used as the containers for the Ottawa sand. After the plants were one inch high, supplementary light from two 60-watt mazda lamps were given each night until harvest.

Six series of boron concentrations were used in the basic nutrient solution: 0.0, 0.001, 0.008, 0.10, 2.5, and 8.0 p.p.m. The six containers receiving

any one of the boron treatments were each given a different indoleacetic acid spray applied to the lettuce, the concentrations being 0.0, 100, 200, 250, and 300 p.p.m., the same solutions used in series I. The boron concentrations used in the two tests varied somewhat, especially in the range of the optimum; but the exact determination of this datum was not one of the principal objects of the study.

Results

Series I plants grew rapidly but owing to the high temperatures or other unknown factors there was little tendency for heads to form. The plants

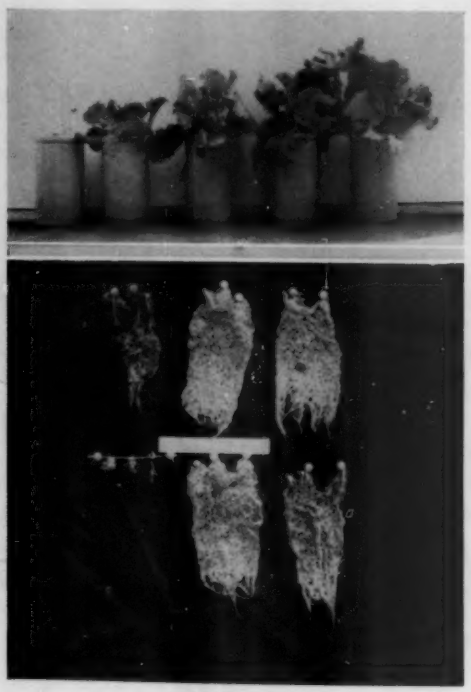


FIG. 1. (Above) Shoot growth in the boron series. Left to right: (1), minus boron, minus indoleacetic acid; (2), minus boron, 300 p.p.m. of indoleacetic acid; (3), 0.1 p.p.m. boron, 300 p.p.m. indoleacetic acid; (4), 5 p.p.m. boron, 500 p.p.m. indoleacetic acid; (5), 0.1 p.p.m. boron, minus indoleacetic acid.

FIG. 2. (Below) Root growth in the boron series. Upper row: shoot given daily spray of 300 p.p.m. indoleacetic acid; from left to right: minus boron, 0.1 p.p.m., and 0.5 p.p.m. boron in the nutrient solution. Lower row without indoleacetic acid; left to right: minus boron, 1 p.p.m., and 0.5 p.p.m. boron in the nutrient solution.

developed a considerable amount of stem and the leaves tended to be less broad than is typical of lettuce grown in the field during cool weather (fig. 1).

As shown in table I, the addition of a very small quantity of boron (0.005 p.p.m.) to the nutrient solution produced a pronounced improvement in the

TABLE I

EFFECT OF BORON CONCENTRATION IN NUTRIENT SOLUTION ON THE GROWTH OF LETTUCE

SHOOT GROWTH

SERIES I

TEST	BORON CONCENTRATION†									
	0.0	0.005	0.025	0.05	0.10	0.25	0.50	1.0	2.5	5.0
Green weight (gm.)*	17.87	55.32	55.76	55.47	56.83	58.23	57.58	55.44	55.57	45.21
Dry weight (gm.)	2.05	4.53	4.42	4.23	4.26	4.43	4.28	4.03	4.07	3.79
Percentage H_2O	88.6	91.8	92.0	92.5	92.6	92.3	92.6	92.7	92.5	91.6

SERIES II

	BORON CONCENTRATION					
	0.0	0.001	0.008	0.10	2.5	8.0
Green weight (gm.)	4.77	14.64	72.9	89.05	108.36	83.37
Dry weight (gm.)	0.45	1.04	3.55	4.66	5.72	4.60
Percentage H_2O	90.8	92.8	95.2	94.6	94.6	94.5

ROOT GROWTH

SERIES I

	BORON CONCENTRATION									
	0.0	0.005	0.025	0.05	0.10	0.25	0.50	1.0	2.5	5.0
Dry weight (gm.)	0.75	1.85	1.71	1.86	1.47	1.93	1.77	1.61	1.52	1.34

SERIES II

	BORON CONCENTRATION					
	0.0	0.001	0.008	1.0	2.5	8.0
Dry weight (gm.)	0.04	0.12	0.21	0.35	0.58	0.28

* Yields are the average of each group yield; a boron group consisting of 7 indoleacetic acid treatments from 0.0 to 300 p.p.m.
† Concentration in parts per million.

fresh weights of the plants. Five parts per million of boron were definitely toxic and caused a marked decline in the fresh and dry weights. Boron in quantities of as much as 2.5 p.p.m. or 5 p.p.m. resulted in death of cells, and production of large brown bands along the margins of the older leaves. The addition of 0.25 p.p.m. of boron to the culture solution appeared to be optimum for the production of green weight of leaves, although the differences in yields of dry or fresh weights for the range of boron applications between 0.005 p.p.m. and 2.5 p.p.m. were not significant.

Without boron added to the nutrient solution there were produced typical, thick, brittle leaves reduced in size and cupped in shape. Brown spots and waxy exudations appeared on the younger leaves, and the growing point gradually became brown and non-functional.

In the series II, where plants were grown in the fall but with supplemental light, there was also little tendency to head; but the plants had shorter stems than did the summer grown plants. The optimum supply of boron for fresh weight and dry weight of shoot material was at a concentration of 2.5 p.p.m., as is shown in table I. Actually the optimum is probably somewhat below 2.5 p.p.m. The differences in yields between the sets of plants supplied with different boron concentrations are all significant in series II. Plants receiving but 0.001 p.p.m. of boron gradually developed deficiency symptoms, especially the cupping of the younger leaves, but these changes did not appear until about 8 days after the first deficiency symptoms appeared on the minus boron plants.

Root growth appeared to be optimum when the concentration of boron in the nutrient solution was 0.25 p.p.m. in series I and 2.5 p.p.m. in series II. Difficulties in obtaining net root weight make this determination very difficult in the case of lettuce.

Roots growing under conditions of boron deficiency were short and stubby with brown, rather than the usual white, growing points. With limited growth, the area for absorption was necessarily decreased although the minus boron plants did not appear to wilt as easily as did the larger plants. The smaller plants, of course, had a relatively greater water supply since all plants were given the same quantity of nutrient solution (fig. 2).

The percentage of water in the shoot tissues of plants of both series was slightly lower in plants grown in solutions with little or no boron supplied. Data showing this trend are given in table I. Other differences in moisture content, except for the decrease in percentage of moisture in plants of series I, with an excess of boron are not of definite significance. It would appear, however, that succulence or tenderness and turgidity of tissue are correlated with optimum nutrition and water supply, which result in rapid growth and large thin walled cells.

The lower concentrations of indoleacetic acid sprayed on the plants of series I daily for the first three weeks produced little change in the growth of the lettuce. Certainly the treatment did not prevent the development of boron deficiency symptoms. The stronger concentrations applied during

† Concentration in parts per million.

the last period of growth caused definite structural peculiarities such as epinasty of the leaves, thicker stems, and lighter colored stems and leaves. The higher concentrations did not prevent the development of boron deficiency symptoms in either series I or II. Although there was some indication of improved growth in series I (minus boron plants treated with indoleacetic acid) this was apparently the result of stronger plants at the beginning, or other factors, as the effect did not appear in the series II test. The data recorded in table II, giving the yields with various concentrations of

TABLE II

EFFECTS OF INDOLEACETIC ACID CONCENTRATION ON THE GROWTH OF LETTUCE

TEST	SHOOT GROWTH						
	INDOLEACTIC ACID CONCENTRATION†						
	SERIES I						
	0.0 D†	0.0 L‡	100	150	200	250	300
Green weight (gm.)§	57.44	58.01	53.15	54.77	55.05	57.02	51.31
Dry weight (gm.)	4.41	4.20	4.29	4.24	4.45	3.91	4.08
Percentage H_2O	92.5	92.5	91.8	92.3	91.9	92.9	92.1
	SERIES II						
Green weight (gm.)	.....	65.82	70.35	60.11	58.6	64.02	54.23
Dry weight (gm.)	.....	3.64	3.83	3.11	3.11	3.45	3.03
	ROOT GROWTH						
	SERIES I						
Dry weight (gm.)	1.53	1.66	1.70	1.56	1.54	1.70	2.02
	SERIES II						
Dry weight (gm.)	.....	0.38	0.32	0.24	0.17	0.18	0.23

§ Yields are the average of each group yield; each indoleacetic group consisted of 10 boron treatments from 0.0 to 5.0 p.p.m.

† D. Distilled water spray.

‡ L. Lanolin emulsion without indoleacetic acid.

* Concentration in parts per million.

indoleacetic acid, show no definite trends which can be ascribed to the auxin. Indoleacetic acid in series II apparently caused a somewhat more rapid elongation of the stems.

Boron deficiency symptoms are different from the so-called tipburn which causes much damage in the field. With the latter malady brown necrotic areas are most often found near the margins of leaves, averaging two or three inches in length. The brownish areas coalesce and tend to spread down the veins a short distance. Young plants, however, often show injury to the growing point as well as farther out on the leaves.

Typical tipburn, near the leaf margin, appeared on the plants of series I which were given boron solutions ranging from .005 to 2.5 p.p.m., but only on plants which did not receive the indoleacetic acid spray. One or two plants which were sprayed with indoleacetic acid in series II showed definite tipburn. In a third series of soil grown plants the tipburn appeared on

leaves sprayed daily with 250 p.p.m. of indoleacetic acid. Sand grown plants did not grow quite rapidly enough to produce the maximum quantity of tipburn.

Summary

1. As little as 0.005 p.p.m. of boron in the nutrient solution gave excellent growth of lettuce and prevented death of cells at the growing points as well as other deficiency symptoms. A concentration of 0.001 p.p.m. of boron delayed the appearance of deficiency symptoms.

2. Indoleacetic acid sprayed on the leaves produced plants with open heads, lighter green color, and shorter and thicker stems in summer grown plants; it did not, however, give evidence of replacing boron in promoting the normal growth and development which plants well supplied with boron have.

3. Neither boron nor indoleacetic acid prevented the appearance of the disorder known as tipburn.

4. The percentage of water in the shoots of the plants grown in a boron deficient medium was slightly lower than it was in the more normal plants grown with sufficient boron. A high boron concentration in the nutrient solution also resulted in a lower moisture content in the plant shoot.

The author wishes to express his appreciation to Dr. E. J. Kraus of the Department of Botany, University of Chicago, for the opportunity to work in that laboratory and to Dr. S. V. Eaton for suggestions during the course of the study.

Colorado State College
Fort Lewis Branch
Hesperus, Colorado

LITERATURE CITED

1. Eaton, F. M. Interrelations in the effects of boron and indoleacetic acid on plant growth. Bot. Gaz. **101**: 700–705. 1940.
2. McHargue, J. S., and Calfee, R. K. Further evidence that boron is essential for the growth of lettuce. Plant Physiol. **8**: 305–313. 1933.
3. McMurtrey, J. E. Distinctive plant symptoms caused by any one of the chemical elements essential for normal development. Bot. Rev. **4**: 183–203. 1938.
4. Minarik, C. E., and Shive, J. W. The effect of boron in the substrate on calcium accumulation by soybean plants. Amer. Jour. Bot. **26**: 827–831. 1939.
5. Schropp, W., and Arenz, B. Versuche mit Bor zu einigen Gramineen. Bodenk. u. Pflanzenernähr. **16**: 169–184. 1940.
6. Shive, J. W. Significant rôles of trace elements in the nutrition of plants. Plant Physiol. **16**: 435–445. 1941.
7. Skoog, Folke. Relationships between zinc and auxin in the growth of higher plants. Amer. Jour. Bot. **27**: 939–951. 1940.

GERMINATION (EMERGENCE) OF VEGETABLE SEED AS AFFECTED BY DIFFERENT SOIL MOISTURE CONDITIONS[1]

L. D. DONEEN AND JOHN H. MACGILLIVRAY

(WITH ONE FIGURE)

Moisture is one essential condition for seed germination, and poor germination is very costly to growers. There is, nevertheless, little experimental evidence concerning the effects of different percentages of available moisture (**5**) on germination; more study of the subject is essential. Since most vegetable seeds are planted at shallow depths, there may be rapid fluctuations of soil moisture around the seed, especially during the warmer months. This condition is common both to the irrigated soils of the west and to the soils of humid areas.

Though several reports have been published concerning the effect of soil moisture upon germination, in no case has there been accurate measurement of the available soil moisture. LIVINGSTON (**3**) indicates that seed germination varies with the species of plant concerned as well as with the percentage of soil moisture. According to COOPER (**2**), several species of forest-tree seeds respond differently to various amounts of supplementary water in addition to natural rainfall. BASS (**1**), studying the germination of New Zealand spinach, found it to be variable; but the germination was increased when the seeds were dried at intervals during the tests. PARKER and OLIVER (**6**) studied the combined effects of soil moisture and fertilizer placement on the germination of cabbage, snap beans, and pea seeds. In some treatments there were indications that low soil moisture and method of fertilizer application reduced germination. MACGILLIVRAY (**4**) has studied the effect of soil moisture, temperature, and varieties of wheat upon germination. Germination was affected by these three factors. As SHULL (**7**) has shown, the seeds of several plant species will absorb water from solutions much higher in atmospheric pressure than 16 atmospheres (**8, 9**) which is approximately the pressure of the permanent wilting percentage (**5**). Xanthium seeds proved able to extract water from a solution whose concentration was 1,000 atmospheres.

Methods and discussion

A Yolo fine sandy loam (field capacity 15.7 per cent., permanent wilting percentage 8.6 per cent.) and a Yolo clay (field capacity 29.9 per cent., permanent wilting percentage 14.9 per cent.), obtained on the University Farm, were used in this experiment because of their different ability to hold the available water. These soils were air dried, and the moisture contents determined. To obtain a series of soils with different moisture percentages, a quantity of soil was placed in a rotating cement mixer and was sprayed with

[1] Mr. EVERETT RYPINSKI and Mr. M. ZOBEL assisted in taking records; Dr. L. D. LEACH cooperated by treating the seed for germination diseases.

TABLE I

GERMINATION (EMERGENCE) OF VEGETABLE SEED IN YOLO FINE SANDY LOAM AT DIFFERENT SOIL MOISTURES, GROUPED WITH REFERENCE TO GERMINATION NEAR PERMANENT WILTING PERCENTAGE (FIELD CAPACITY 15.7 PER CENT.; PERMANENT WILTING PERCENTAGE 8.6 PER CENT.)

SEEDS TESTED	PERCENTAGE GERMINATION BY OFFICIAL METHODS	PERCENTAGE SOIL MOISTURE									LEAST SIGNIFICANT DIFFERENCE
		7	8	9	10	11	12	14	16	18	
		GERMINATION									
	%	%	%	%	%	%	%	%	%	%	
Group A											
Cabbage, Copenhagen Market	93	0	80	94	95	92	93	93	91	86	7.7
Sunflower, dwarf	.....	0	73	89	90	90	92	92	82	90	9.2
Turnip, Purple Top White Globe	92	0	72	89	88	91	91	87	90	87	7.7
Radish, Scarlet Globe	95	0	64	94	89	95	92	95	94	90	9.7
Summer squash, Zucchini	98	0	31	98	98	99	99	99	98	97	2.3
Sweet corn, Golden Bantam	95	2	35	90	95	93	93	89	93	95	6.9
Watermelon, Striped Klondike (Blue Ribbon)	86	1	39	82	83	83	84	87	85	85	8.8
Tomato, Essar	97	0	31	79	88	95	93	95	91	93	8.4
Winter squash, Hubbard	99	1	22	86	94	93	96	96	95	96	8.9
Cantaloupe, Mildew Resistant #45	99	0	7	92	99	97	99	97	97	96	3.7
Pepper, Pickling Wax	89	0	19	75	75	73	76	79	80	74	9.6
Cucumber, Short Colorado	90	0	0	84	97	99	98	98	99	98	4.7
Onion, Yellow Sweet Spanish	96	0	0	75	90	91	90	91	91	91	7.6
Spinach, Viroflay	87	0	0	74	90	93	94	94	95	85	6.1
Group B											
Carrot, Imperator	91	0	3	57	75	87	76	78	77	78	10.4
Snap beans, Stringless Greenpod	82	0	0	57	80	86	92	89	88	89	7.5
Spinach, New Zealand (seed balls)	.....	..	4	42	64	76	81	83	80	61	8.7
Spinach, New Zealand (seedlings)	.....	0	5	63	87	117	119	141	132	96	16.6
Group C											
Lettuce, Hanson	93	0	0	29	65	81	91	91	90	88	8.2
Lima beans, Baby Potato	88	0	0	23	79	89	86	86	89	91	5.5
Peas, Laxton's Progress	91	0	3	19	73	86	87	89	86	90	16.6
Beets, Detroit Dark Red (seed balls)	91	0	3	4	52	82	90	91	93	92	6.0
Beets, Detroit Dark Red (seedlings)	.....	0	3	5	77	129	156	167	179	172	32.7
Group D											
Celery	80	0	0	0	0	29	43	62	73	82	.....

sufficient water to bring it up to the desired moisture level. In the case of the Yolo fine sandy loam it was possible to bring and hold the soil moisture above the moisture equivalent (18 per cent., table I). Some puddling of the soil was noted at this moisture content. By this method it was possible to obtain, within the limits of $\pm$ 0.2 per cent., the soil moisture desired. At and above 10 per cent. soil moisture for Yolo fine sandy loam and 20 per cent. soil moisture for Yolo clay, some of the crop seeds were treated with suitable seed disinfectants to obtain maximum germination. Seed treatments were not used at low soil moistures because they reduced the germination. With most of the crops 350 grams of the soil was placed in a no. 2 friction-top can, the soil was tamped, and 10 seeds were spaced evenly on the soil surface. Fifty grams of soil were used to cover the seed, and the surface again tamped. The larger seeds were placed on 300 gm. of soil and covered with 100 gm., so that in all cases each can held 400 gm. Twenty cans, or 200 seeds, were used for the averages of the Yolo fine sandy loam, and ten cans with 100 seeds for the Yolo clay. The seeds in these cans were germinated in a constant-temperature room at 77° F. except celery, lettuce, onions, and peas which were placed at 64° F. "Germination" as used in this paper indicates the percentage of seeds that sprouted and from which the plant emerged above the soil surface. Germination was recorded daily from the second to ninth day after seeding, and every other day for the remainder of the three-week germination period.

To obtain satisfactory germination, the lettuce seeds were planted $\frac{1}{16}$ of an inch deep in the soil, but otherwise were handled in the usual way. Celery did not give satisfactory germination under the same conditions, but germinated well when planted in the usual soil mixture in common Petri dishes. One hundred seeds were planted in each dish. For celery, better germination than that shown in table I could be obtained by adding a drop of water to each seed after planting or by covering the soil surface with a wet filter paper or a wet cloth. Under these conditions 100 per cent. germination was obtained in a single Petri-dish trial.

At the end of the experiment all cans were screened so the seeds could be examined and the sprouting noted. Sometimes the percentage of moisture was determined after screening. For beets and New Zealand spinach, both the germination of the seed balls and the number of seedlings per 100 seed balls were recorded. Sunflower seeds were included with the vegetables because they are frequently used in determining the permanent wilting percentage.

Based on these data, the vegetable crops seem to fall into four groups. The classification was made by using the percentage germination just above the permanent wilting percentage; in other words, 9 per cent. for the loam and 16 per cent. for the clay soils. Thus in group A the total germination covered a range of 98 to 74; in group B, 57 to 42; in group C, 29 to 4; and in group D, 0. Winter squash in table II was the only vegetable that failed to meet these criteria.

Vegetable seeds give a satisfactory field germination percentage over the entire range of available soil moisture except for lima beans, beets, celery, lettuce, peas, and New Zealand spinach, which germinated less than 50 per cent. near the permanent wilting percentage. In addition, snap beans, carrots, and winter squash (clay soil only) seeds germinated less than 70 per cent. With some of the crops there was considerable germination in both soils below the permanent wilting percentage. The germination at low soil moisture appeared not to be correlated with the size of seeds.

The germination of celery seeds was very striking because none was obtained below 11 per cent. soil moisture, which was well above the permanent wilting percentage. This likely accounts for a common greenhouse practice

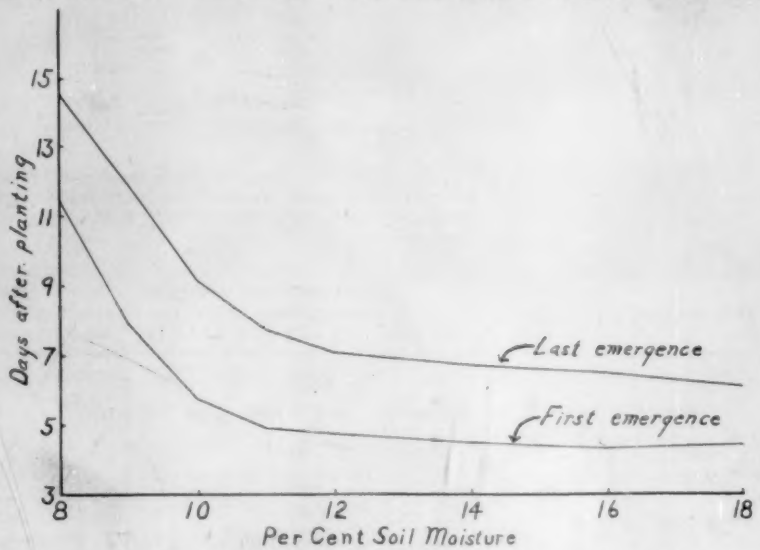


FIG. 1. Interval in days between planting and emergence of first and last seeding. Average of fifteen crops planted in Yolo fine sandy loam.

of starting celery plants by scattering the seeds on the surface of the soil in a greenhouse flat, and then screening soil on the seeds until about half of them are covered. To insure high moisture, the flat is watered frequently through a piece of burlap sack and may also be covered with a piece of window glass. By keeping the burlap wet, the seeds are germinated in wet soil at a high humidity.

Seeds germinated more quickly at high soil moistures than at low and, except for the 8 per cent. soil moisture, the interval between first and last germination increased as the water content decreased. All crops that germinated at 8 per cent. soil moisture and above were used for figure 1. The soil moisture was determined in several cans of 9 per cent. and 10 per cent. series after the germination period of three weeks, to determine whether all the available moisture had been absorbed by the seeds. For a representative sample of the crops, the soil moisture was reduced below the permanent

wilting percentage in 38 per cent. of the cases in the 9 per cent. soil moisture, and in only 13 per cent. of the cases in the 10 per cent. soil moisture (table I). In all trials in this work, the soil moisture given is that of the soil at the time of seed planting, and the reduction in soil moisture was critical only at low soil moistures.

Though the seeds of certain crops had sprouted at the low soil moistures, the plant did not appear above ground and so was not counted in the germination. Crops that germinated less than 80 per cent. at 9 per cent. soil moisture and that had a fair percentage of sprouted seed not appearing above the surface were lima beans (61 per cent.), beets (31 per cent.), and peas (68 per cent.). Lettuce had only 3 per cent. sprouted seed at 9 per cent. soil moisture. Many of these crops germinated less at the highest soil moistures than at some lower moisture, but the differences were significant only for spinach and New Zealand spinach.

TABLE II

GERMINATION (EMERGENCE) AT 77° F. OF VEGETABLE SEEDS IN YOLO CLAY SOIL AT DIFFERENT SOIL MOISTURES, GROUPED WITH REFERENCE TO GERMINATION NEAR PERMANENT WILTING PERCENTAGE. (FIELD CAPACITY = 29.9 PER CENT.; PERMANENT WILTING PERCENTAGE = 14.9 PER CENT.)

SEEDS TESTED	OFFICIAL GERMINATION	PERCENTAGE SOIL MOISTURE								LEAST SIGNIFICANT DIFFERENCE
		14	16	18	20	22	24	26	28	
		GERMINATION								
	%	%	%	%	%	%	%	%	%	
Group A										
Corn, sweet	95	38	80	95	91	87	95	91	90	3.4
Cucumber	90	30	91	90	85	88	84	82	84	19.9
Radish	95	2	85	96	93	93	95	95	92	8.2
Squash, winter	99	0	55	71	63	79	85	81	82	26.2
Tomato	97	11	93	89	91	95	89	91	87	8.4

Germination in closed tin containers was probably more favorable for some seeds than for others. All seeds germinated within 5 per cent. of the official germination except carrots, onions, and peppers for the sandy loam; and winter squash for the clay soil. In preliminary trials there was some damping-off or rotting of seedlings in the cans. This was reduced and greater germination obtained by proper seed treatments; but sterilized soil was not used because steam sterilization may affect the moisture-holding capacity of the soil.

For statistical analysis the percentage of germination was calculated on the basis of the 10 seeds in each can. At first it was planned to use 10 cans of each vegetable and each treatment. Since, however, the results were variable, the number of cans was increased to 20. Even then the errors were much greater for some vegetables than for others. The large error for cucumbers and winter squash on the clay soil (table II) is probably due in part to the use of only 100 seeds in 10 cans.

With the normal procedure it was impossible to obtain satisfactory germination of lettuce, celery, endive, and parsnips. Some of these crops have been mentioned already. Endive gave as high as 62 per cent. germination (official germination, 95 per cent.) at the higher figures of 14 to 18 per cent. soil moisture. Parsnip seeds gave poor germination at all soil moistures in cans; the official germination was not known. Endive and parsnips were not included in the tables; they may be typical of seeds found in group C or D.

Summary

Most vegetable seeds gave good germination over the entire range of available water and seem to fall into four groups based on their ability to germinate near the permanent wilting percentage. Seeds of all crops germinated in a shorter time at high soil moistures than at low.

DIVISIONS OF IRRIGATION AND TRUCK CROPS
UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA

LITERATURE CITED

1. BASS, C. M. Methods of germinating New Zealand spinach. Proc. Assoc. Off. Seed Anal. No. Amer. Twenty-first Ann. Meet., pp. 36–37. 1929.
2. COOPER, J. R. Forty-third Ann. Report. Arkansas Agr. Exp. Sta. Bull. 268. 1931.
3. LIVINGSTON, B. E. The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Washington Pub. no. 50: 40–41. 1906.
4. MACGILLIVRAY, JOHN H. The effect of soil moistures and of temperatures on the germination and growth of varieties of wheat. Thesis, Graduate School, University of Illinois, 1922.
5. ————, and L. D. DONEEN. Soil moisture conditions as related to the irrigation of truck crops on mineral soils. Proc. Amer. Soc. Hort. Sci. **40**: 483–492. 1942.
6. PARKER, M. M., and R. C. OLIVER. The effect of fertilizer placement, as influenced by soil moisture, on seed germination. Proc. Amer. Soc. Hort. Sci. **36**: 533–536. 1939.
7. SHULL, C. A. Measurement of surface forces in soils. Bot. Gaz. **62**: 1–31. 1916.
8. VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil moisture at permanent wilting of plants. Plant Physiol. **3**: 355–357. 1928.
9. ————, and ————. Some plant and soil moisture relations. Amer. Soil Sur. Assoc. Bull. **15**: 76–80. 1934.

INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON THE DEVELOPMENT OF LEAFY MOSS PLANTS[1]

SAMUEL L. MEYER AND CLIFFORD H. FORD

Introduction

The literature on the physiology of mosses contains interesting results of investigations concerning the influence of the hydrogen-ion concentration of the substrate on the germination of moss spores, the growth of protonemata, and the development of leafy plants. In many cases, the conclusions reached are in marked disagreement.

TREBOUX (**7**) observed that the spores of such acid substrate mosses as Sphagnum and Dicranella germinate best in an acid medium. KESSLER (**3**) reported that the spores of mosses which grow under basic conditions germinate on an alkaline substrate while spores of mosses which grow in an acid environment germinate on a medium of similar reaction. PRINGSHEIM (**5**) concluded that though the protonemata of *Leptobryum piriforme* grow best in a slightly acid solution buds develop best in an alkaline medium. SCHWEIZER (**6**) obtained some interesting results with *Funaria hygrometrica*, a species which naturally occurs on alkaline or only slightly acid substrates. He found that spores can withstand a pH of 1.55 without impairment of germination; that protonemata develop equally well whether the acidity of the medium is high or low; and that young moss plants develop equally well in cultures of high or low acidity. IKENBERRY (**2**) studied the hydrogen-ion concentration of the habitats of various moss species as well as the pH values of solutions most favorable to spore germination and development of protonemata. He concluded that: "In general, there is no consistent correlation between the pH of the substratum where a given species is found in greatest abundance in nature and the reaction of the culture solution in which its spores germinate and protonema grow most readily." APINIS (**1**) observed that in order for protonemata to give rise to leafy plants: "It is necessary that the pH of the solution correspond to the pH interval observed in natural conditions." He concluded that in *Sphagnum plumosum*, *Funaria hygrometrica*, and *Polytrichum juniperinum:* "It was experimentally found that the pH interval of the leaf-bearing moss development or its optimum corresponds to the acidity of the substratum of the respective mosses in natural conditions." This conclusion is of particular interest when compared with the results of SCHWEIZER (**6**), mentioned above. APINIS cited the work of TREBOUX (**7**), KESSLER (**3**), PRINGSHEIM (**5**), and IKENBERRY (**2**) but apparently overlooked the contribution of SCHWEIZER (**6**). APINIS, however, very aptly summed up the

[1] Contributions from the Botanical Laboratory, The University of Tennessee, n. ser. no. 65. This is the fifth of a series of studies on the physiology of mosses by the senior writer.

entire situation when he said: "It is apparent from the above that there is a certain uncertainty about this matter."

The present investigation was begun in order to determine the relation, if any, between the hydrogen-ion concentration of the substrate and the development of leafy moss plants from primary protonemata.

Materials and methods

For this investigation, eight buffered culture solutions were prepared in the manner described by IKENBERRY (2). A nutrient solution was prepared with the following composition: $Ca(NO_3)_2 \cdot 4H_2O$, 0.491 gm.; $MgSO_4 \cdot 7H_2O$, 0.056 gm.; $Fe_2(SO_4)_3$, trace; and distilled water to 1000 ml. To a given amount of the nutrient solution, an equal quantity of phosphate buffers was added. The latter were combined in various proportions to give solutions of different pH values. The phosphate buffer mixtures were prepared from the following stock solutions: H_3PO_4, 2.178 gm. per liter; KH_2PO_4, 3.026 gm. per liter; K_2HPO_4, 3.872 gm. per liter; and K_3PO_4, 4.718 gm. per liter. A solid substrate nutrient medium was prepared by using the above mentioned solutions and 2 per cent. washed agar.

Eight series of cultures were set up in 125-ml. Erlenmeyer flasks, each flask containing 50 ml. of the buffered nutrient agar. Five flasks were included in each series. A Youden quinhydrone pH apparatus was used in making the pH determinations. The pH value of the cultures in each series is shown below:

SERIES	pH VALUE	SPECIFIC ACIDITY
I	4.28	525.0
II	5.94	11.5
III	6.21	6.17
IV	6.46	3.47
V	6.99	1.02
VI	7.51	0.309
VII	7.70	0.20
VIII	7.97	0.107

Funaria hygrometrica was the species used in these experiments. It was used by APINIS (1) and SCHWEIZER (6). Spores were germinated on the liquid nutrient solution in a Petri dish. After germination, and when the protonemata were long enough to be handled easily, protonemata from several spores were transferred to each flask by means of a fine glass needle.

The cultures were kept at room temperature and the source of illumination was light from a laboratory window.

Observations and discussion

Several studies have been made of the hydrogen-ion concentration of the substrate on which *Funaria hygrometrica* occurs in nature. MONTGOMERY (4) listed the occurrence of that species in a pH range of 5.2 to 7.6. His observations were based on two tests. IKENBERRY (2) found *Funaria hygro-*

metrica in a range of slightly less than pH 7 to pH 9, most frequently around pH 8. His conclusions were based upon 56 separate collections. APINIS (**1**) found that the same species grew in a pH range of 5.8 to 8.4, usually in pH 6.0 to 8.0. He also observed that though the spores germinated and protonemata developed in a pH range of 4.5 to 9.0, "Moss development is detected only on neutral and alkaline substrata pH 7.1 to 8.8." These pH values, indicating the range in which leafy plants were formed most abundantly, correspond closely to the pH value reported by IKENBERRY (**2**) for most frequent occurrence of that species (pH 8). They appear to support the conclusion of APINIS (**1**) that there is a correlation between the pH of the substrate where a species of moss occurs in nature and the pH of the artificial medium on which protonemata develop leafy plants.

The pH values of the culture series used in this investigation ranged from approximately 4 to 8. Some of these values are considerably more acid than those of the substrates on which *Funaria hygrometrica* has been found growing in nature. The pH values of series I through series V, pH 4.28 to 6.99, are much more acid than the neutral or alkaline substrates of pH 7.1 to 8.8 on which the development of leafy plants is reported to have been limited in the investigations of APINIS (**1**).

Final observations were made at the conclusion of a growing period of approximately three months. Protonemata grew and leafy plants developed in cultures at every pH value. These results indicate that protonemata of *Funaria hygrometrica* produce leafy plants on substrates that are considerably more acid in reaction than those on which that species usually occurs in nature. Furthermore, the pH values of the media on which leafy plants develop are much more acid than the limited range in which that development is reported by APINIS (**1**).

These observations support the general conclusion of SCHWEIZER (**6**) that leafy plants of *Funaria hygrometrica* develop on substrates of both high and low acidity though the cultures used were mostly in the acid range of pH values. No evidence has been found to support the experimental results of APINIS (**1**) or his suggestion relative to a possible relation between the pH of the substrate where a moss species occurs in nature and the pH range of the artificial media on which leafy plants develop.

Summary

Results of investigations with *Funaria hygrometrica* grown from primary protonemata on buffered nutrient agar substrates of pH values ranging from 4.28 to 7.97 indicate that there is no clearly defined correlation between the pH value of the substrate on which that moss species most frequently occurs in nature and the pH value of the artificial media on which leafy plants develop.

DEPARTMENT OF BOTANY
THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE

LITERATURE CITED

1. APINIS, A. Data on the ecology of bryophytes III. The significance of hydrogen-ion concentration on germination of spores and development of some mosses. Acta Horti Bot. Univ. Lat. **11/12**: 1–22. 1939.
2. IKENBERRY, G. J. The relation of hydrogen-ion concentration to the growth and distribution of mosses. Amer. Jour. Bot. **23**: 271–279. 1936.
3. KESSLER, B. Beiträge zur Ökologie der Laubmoose. Beih. bot. Centralblatt. **31**: 358–387. 1914.
4. MONTGOMERY, C. E. Ecology of the mosses of the Grand de Tour region of Illinois, with special reference to hydrogen-ion relations. Bot. Gaz. **91**: 225–251. 1931.
5. PRINGSHEIM, E. G. Physiologische Studien an Moosen. 1 Mitt. Die Reinkultur von *Leptobryum piriforme* (L.) Schpr. Jahrb. wiss. Bot. **60**: 499–530. 1921.
6. SCHWEIZER, G. Physiologisch-morphologische Studien über *Funaria hygrometrica* L. in Reinkultur. Ber. d. bot. Ges. **48**: 75–84. 1930.
7. TREBOUX, O. Die Keimung der Moossporen in ihrer Beziehung zum Lichte. Ber. d. bot. Ges. **23**: 397–401. 1905.

BRIEF PAPER

BLOSSOM INDUCTION OF THE CRANBERRY

R. H. Roberts and B. Esther Struckmeyer

(with two figures)

As part of a study of the growth and fruiting habits of the cranberry (3) data were collected to determine the time of blossom induction. The method used was similar to that employed in finding the time of induction of the

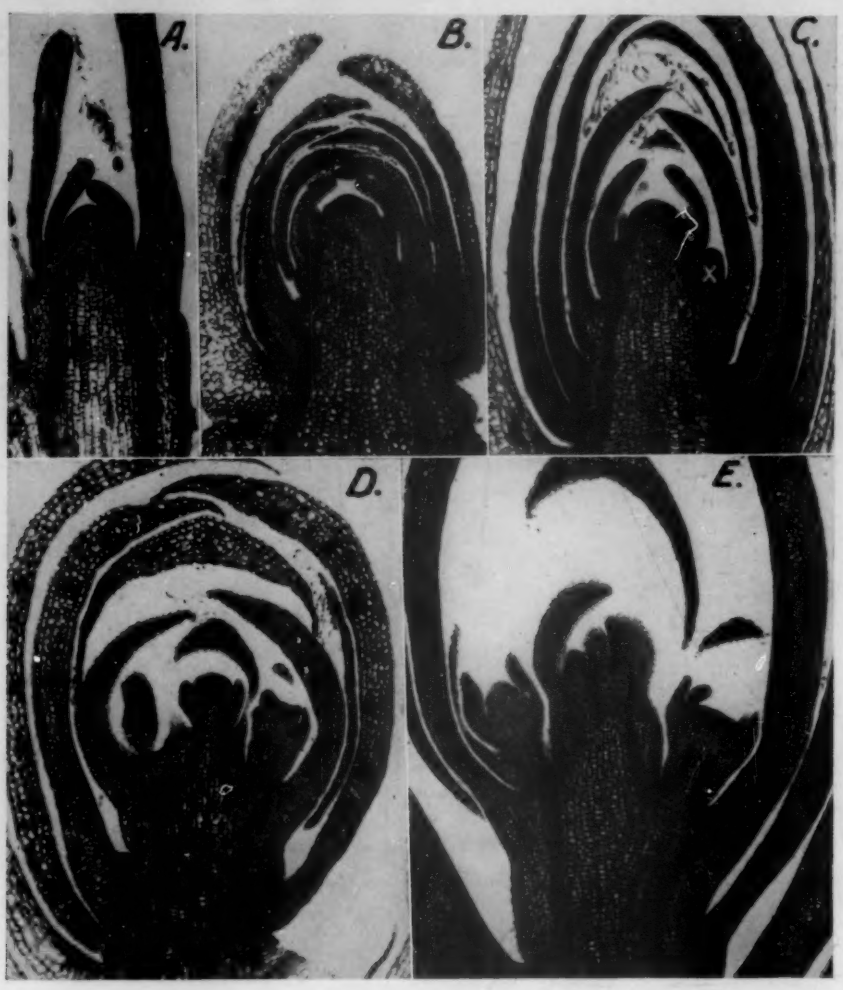


Fig. 1. Cranberry blossom initiation, 1942 variety McFarlin. A. Tip of vegetative upright, June 19. B. Terminal bud, July 21. C. Blossom primordia, at cross, July 29. D. Blossom development, August 17. E. Same, September 8.

apple (4). Twenty-five to thirty uprights were defoliated (except the 2 or 3 terminal leaves) at 5- to 8-day intervals from the time the earliest blossoms hooked (turned down), about June 4, until three to four weeks after shoot growth was completed, on August 13.

An exact dating of induction is difficult because of the range of development which is typical of the cranberry. For example, the blossom period extended from June 10 until July 21 and the period of full blossom from June 25 to July 8. The growth of non-blossoming uprights was completed about July 20 as determined by averaging the lengths of 60 to 75 new growths weekly. Many new growths would have been through elongating 10 to 15 days previously.

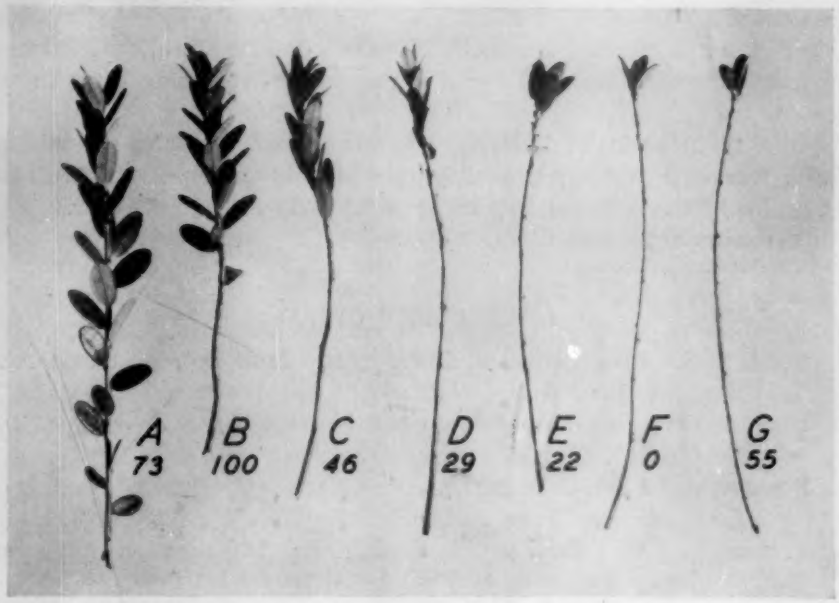


FIG. 2. Samples of uprights photographed September 16. Numbers show percentages of blossom buds formed. A. Untreated upright. B. Defoliated, June 4. Much new growth added. C. Defoliated, June 10. D. Defoliated, June 18. E. Defoliated, June 25. Some new leaves formed after defoliation. F. Defoliated, July 8. No new leaves. G. Defoliated, July 21. The presence of blossom buds on these shoots with no new leaves indicates induction was under way prior to defoliation.

The first blossom primordia on untreated uprights were found July 29 (fig. 1, C). This compares well with the observations of GOFF in 1901 (1) and those of LACROIX (2) on plants growing in Massachusetts.

The defoliated uprights were collected September 16 and the tips examined for blossom primordia. Representative shoots are shown in figure 2. Elongating growth and new leaves were formed following leaf removals until June 25. These new leaves induced blossom buds. Defoliation on July 8 inhibited bud formation (except on the few uprights upon which a

second growth and new leaves were produced following leaf removal). Many of the uprights which were defoliated July 15 differentiated blossom buds. That is, removal of the leaves on this date did not prevent bud formation. This is taken as evidence that induction was under way prior to this time. Uprights which were defoliated at later successive intervals showed increasing percentages of blossom buds. The dates of defoliation and percentages of blossoms formed follows (see fig. 2): June 4, 100.0; June 10, 45.5; June 18, 28.5; June 25, 21.5; June 30, 12.5; July 8, 0.0; July 15, 38.4; July 21, 54.5; July 29, 59.1; August 4, 72.7; and August 13, 80.0. Undefoliated uprights had 72.1 percent. of terminal blossom buds. The reason for the lower percentage of blossom bud formation on the check uprights than on the earlier defoliated ones and on the last ones to be treated, was because the checks included some later-starting and less vegetative shoots than those selected for the defoliation tests. Only the more prominent, and so, more vigorous growths were used for leaf removals, particularly early in the season.

In 1942, induction of McFarlin cranberry blossom buds at Black River Falls, Wisconsin, was approximately July 10 and the earliest observed blossom primordia were found July 29, approximately three weeks later.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. GOFF, E. S. Investigation of flower-buds. Ann. Rpt. Wisconsin Agr. Exp. Sta. **18**: 304–319. 1901.
2. LACROIX, D. S. Cranberry flower-bud investigations. Jour. Agr. Res. **33**: 355–363. 1926.
3. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER. Growth and fruiting of the cranberry. Proc. Amer. Soc. Hort. Sci. **40**: 373–379. 1942.
4. STRUCKMEYER, B. ESTHER, and ROBERTS, R. H. Investigation on the time of blossom induction in Wealthy Apple trees. Proc. Amer. Soc. Hort. Sci. **40**: 113–119. 1942.

NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists has resulted in the naming of Dr. B. S. MEYER of The Ohio State University as president for 1943–1944. Dr. PAUL J. KRAMER, Duke University, is vice-president; and Dr. EARL S. JOHNSTON, The Smithsonian Institution, Washington, D. C., has been chosen secretary-treasurer. A tie vote for one of the offices will have to be decided by the executive committee.

Sectional Meetings.—One after the other, the regional sections have found it impossible to hold the meetings which would have been held in 1943. The Southern Section usually meets in February with the Southern Agricultural Workers, but the latter group held no meeting. The New England Section has its annual meeting in May, but postponed meeting again until conditions for travel are more favorable. The Western Section had planned a meeting at Corvallis, Oregon, but rationing and transportation problems led to the cancellation of the meeting. It seems probable now that no meetings can be held until the restrictions upon such activities are relaxed. In the meantime the American Society of Plant Physiologists and its Sections will find it necessary to transact business. The constitutional provisions should be followed strictly where provisions exist; and where they do not, steps should be taken to provide the necessary machinery for the transaction of business. The constitution of the parent society, By-Laws Section 2 a, provides that: "The executive committee shall have power to consider and act upon all matters not reserved to the Society as a whole." Steps should be taken at once by the executive committee to prescribe the methods to be employed during this meetingless interim. It would be unfortunate to allow irregularities to creep into our transaction of any required business.

On May 7, 1943, the American Society of Plant Physiologists lost one of its oldest members by death. Dr. ALEXANDER PIERCE ANDERSON of Red Wing, Minnesota, passed his eightieth birthday anniversary on November 22, 1942. He became a member of the Society not long after it was organized, his name appearing in the second membership list issued in the spring of 1927. He was a staunch supporter of the Society, having been one of the generous donors who helped to found the STEPHEN HALES award in 1927. Later on he became one of the small list of patrons (the second of them to be deceased), and in 1937 he was honored at Indianapolis by being awarded a CHARLES REID BARNES life membership.

A brief biography of Dr. ANDERSON was published in PLANT PHYSIOLOGY **13**: 214–215. 1937, so that it is not necessary to repeat the details of his life work. He was a fellow of the American Association for the Advance-

ment of Science, a member of the American Forestry Association, the Minnesota Academy of Sciences, the Minnesota Historical Society, and the Geological Society of Minnesota. His experimental work on cereal foods was very extensive, and embraced over 15,000 experiments.

To all surviving members of his family we extend our sympathy. If man's greatness is in proportion to the extent of his service to all, Dr. ANDERSON was truly a great man; for his work contributed to the pleasure and welfare of the whole world.

Tissue Culture.—An unusually fine work has come to the attention of plant physiologists in PHILIP R. WHITE's *A Handbook of Plant Tissue Culture*. It lives up to its name, as it is a small volume, a genuine handbook, and the subject is presented with a fulness and clarity that one seldom finds in a brief book. Dr. WHITE is a gifted writer, and it is a great service that he has rendered in the preparation of this work. There are ten chapters in all: The introduction, setting forth the problems of morphogenesis and the origin of functions; the history of plant tissue culture; the living materials; the laboratory; nutrients; how cultures are started; culture techniques; growth measurements and their interpretation; tissue culture and the study of problems in the pathology and general physiology of plants; and morphogenesis. A bibliography of 457 titles, and a general index complete the work. It is well illustrated, with 71 figures. One of the appreciated features is the series of portraits of the investigators who have contributed much to the field. The detailed description of the laboratory, and the meticulous directions for manipulation of apparatus, the making of media, recording results, interpreting and applying the techniques to new problems will be extremely helpful to anyone interested in doing work with tissue cultures. It is published by the Jaques Cattell Press, Lancaster, Pennsylvania, at $3.75 per copy. While the book has 276 pages, the text occupies but 226 pages. The print is large and clear, the pages small, and one can read all of it in a few hours. It should find a place in every physiologist's library.

Enzymes.—A good book on enzymes for the student and investigator comes from the Cornell University Biochemistry Department. It is entitled *Chemistry and Methods of Enzymes*, and the authors are Dr. JAMES B. SUMNER and Dr. G. FRED SOMERS. It is an excellent general survey, without too much detail. It is divided into four parts, the first of which is an introductory chapter on the general properties of enzymes. Part 2 is devoted to the hydrolytic enzymes, seven chapters, as follows: Esterases; carbohydrases; enzymes of carbohydrate metabolism; nucleases; amidases; and proteolytic enzymes. Part 3 considers the oxidative enzymes. The chapter headings are: Oxidizing enzymes; the iron enzymes; the copper enzymes; dehydrogenases containing coenzymes 1 and 2; oxidases which transfer

hydrogen to cytochrome; the yellow enzymes; nuclein deaminases; miscellaneous oxidases; and desmolases.

Part four discusses the following: Hydrases and mutases; and carbohydrate metabolism. There are author and subject indexes, also a short history of enzymes down to 1931, and a literature list just preceding the introduction. The history is told in less than thirty events which were milestones of progress during about 150 years. Each chapter is closed by a list of pertinent references. The reviewer is very favorably impressed with the work, and it ought to help clarify what has become a very difficult field if all of the details are considered.

It is of modest size, only 365 pages, and the price per copy is $5.00. The publishers are Academic Press, Inc., 125 East 23rd St., New York, N. Y.

Vitamins and Hormones.—The first volume of an annual series on vitamins and hormones bears this title. The editors are Dr. ROBERT S. HARRIS and Dr. KENNETH V. THIMANN, of Harvard University. It bears a foreword by Dr. E. V. MCCOLLUM of Johns Hopkins University. There are 10 reviews, with the following titles and authors: Choline—chemistry and significance as a dietary factor, by C. H. BEST and C. C. LUCAS; the appraisal of nutritional states, by NORMAN JOLLIFFE and RITA M. MOST; physical methods for the identification and assay of vitamins and hormones, by JOHN F. LOOFBOUROW; the chemical and physiological relationship between vitamins and amino acids, by H. H. MITCHELL; the photoreceptor function of the carotenoids and vitamins A, by GEORGE WALD; the significance of the vitamin content of tissues, by ROGER J. WILLIAMS; growth-factors for protozoa, by RICHARD P. HALL; physiology of anti-pernicious anemia material, by GEORGE R. MINOT and MAURICE B. STRAUSS; the intermediate metabolism of the sex hormones, by GREGORY PINCUS and WILLIAM H. PEARLMAN; and the hormones of the adrenal cortex, by T. REICHSTEIN and C. W. SHOPPEE.

The work constitutes a review of certain aspects of biochemistry. Such works have become a necessity to the busy investigator, and for those working on vitamins or hormones, or for those who desire technical information on the subjects covered. The value of the reviews depends upon the thoroughness with which the ground is covered, and the skill of the reviewers in summarizing and interpreting the trends of research. This volume seems to have been carefully prepared, and we hope that the editors and publishers succeed in establishing the series as an annual service.

This first volume contains 452 pages, and its list price is $6.50. The publishers are Academic Press, Inc., New York, N. Y.

Annual Review of Biochemistry.—Volume XII of this annual is of more than usual interest to the plant physiologist because of the large number of the reviews that fall more or less directly in the field of plant physiology. Naturally some of the reviews are in the animal field, but every physiologist finds it valuable to know something of both animal and plant

physiology. One cannot do justice to these fine reviews because of the number and variety of subjects covered. It is possible, however, to list them so that prospective purchasers will know the nature of the volume. There are 24 reviews, with the following titles and authors: Biological oxidations and reductions, by F. LIPMANN; proteolytic enzymes, by R. M. HERRIOTT; the steroids, by H. SOBOTKA and E. BLOCH; the chemistry of the proteins and amino acids, section I by L. F. HEWITT, and section II by R. A. KEKWICK and A. S. MCFARLANE; the chemistry and metabolism of the compounds of sulphur, by J. C. ANDREWS; carbohydrate metabolism, by H. J. DEUEL, JR.; fat metabolism, by G. O. BURR and R. H. BARNES; the metabolism of proteins and amino acids, by H. BORSOOK and J. W. DUBNOFF; the chemistry of the carbohydrates, by H. S. ISBELL; the chemistry of the lipins, by S. J. THANNHAUSER and G. SCHMIDT; mineral nutrition, by L. A. MAYNARD and J. K. LOOSLI; the chemistry of the hormones, by H. FRAENKEL-CONRAT; water-soluble vitamins, by R. J. WILLIAMS; fat-soluble vitamins, by K. HICKMAN; nutrition, 1941 and 1942, by C. S. LANGFORD and H. C. SHERMAN; animal pigments, by C. RIMINGTON; synthetic drugs, by T. C. DANIELS; photosynthesis, by E. S. JOHNSTON and J. E. MYERS; mineral nutrition of plants, by D. I. ARNON; carbon dioxide assimilation in heterotrophic organisms, by C. B. VAN NIEL; the electron microscope in biology, by L. MARTON; the chemistry of viruses, by C. L. HOAGLAND; and microchemistry, by A. A. BENEDETTI-PICHLER. The customary author and subject indexes close the volume.

This series of volumes has been successful, no doubt beyond all anticipation by the editors when they first projected the series. It has been growing in importance and value with the years, until now it constitutes the best source of information biochemists have aside from the original papers on which the reviews are based. The latter are frequently not available, and the reviews constitute an outstanding service to all investigators. If a worker in biochemistry could have but one series of volumes at his side, it should be a complete file of the Annual Review of Biochemistry.

The price is still only $5.00, and orders for it should go to Annual Reviews, Inc., Stanford University P.O., California.

Minor Elements.—The Fourth Supplement to the Third Edition of the well-known *Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition* has been published by the Chilean Nitrate Educational Bureau, Inc., under the direction of HERBERT C. BREWER. It contains 92 pages, and is arranged in the same manner as previous supplements. More than half of the known elements are included, and some 900 or more authors have investigated the elements in connection with more than 100 crop plants.

The value of this annotated bibliography to plant physiologists and agricultural workers has been amply demonstrated. It is the best example of bibliographic service we have, and is highly appreciated by students and

investigators alike. For copies, write to HERBERT C. BREWER, Director, The Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York, N. Y.

Evaporation and Transpiration.—Publication no. 550 of the Carnegie Institution of Washington, Washington, D. C., is entitled: *Studies of Evaporation and Transpiration under Controlled Conditions.* The author is EMMETT MARTIN. It is a critical study of the quantitative aspects of evaporation and transpiration to test the adequacy of LEIGHLY's formula, which was found to be inadequate. Using blotting-paper evaporimeters of various sizes and shapes, MARTIN has found that the rate of evaporation per unit area of surface varied directly with the square root of the wind velocity, inversely with the 0.3 power of the dimension parallel to the wind flow, and inversely with the 0.2 power of the dimension at right angles to wind flow.

Studies were also made of the correlation between the rate of transpiration and relative humidity at three temperatures, 27°, 38°, and 49° C., with ample soil moisture. Young plants showed approximately linear relations except at 49°, at which temperature the rates at low relative humidities tended to run below the expected values. Older plants showed less transpiration at 27° than younger plants. At 38°, the rates were lower in older plants than young only when the humidities fell below 50 per cent. At 49°, the difference between old and young plants disappeared, presumably because of changes in the permeability of protoplasm conditioned by age and temperature.

The effects of wind on transpiration rates increased with temperature increase, probably because of increasing cuticular transpiration. Wind velocity of 250 cm./sec. at high temperatures and low relative humidities sometimes resulted in closure of stomata in less than 3 minutes.

The author studied the ratios of nighttime to daytime transpiration rates, the temperature depression of leaves (20° maximum depression), the effects of radiation on the transpiration rate, the importance of leaf size, and the rate of energy exchange under different conditions.

It is an interesting and valuable contribution to the dynamics of evaporation and transpiration, and the factors which modify plant behavior. Copies may be obtained from The Carnegie Institution of Washington, Washington, D. C.

Soil Science.—*The Fundamentals of Soil Science* is a text book on soil science by C. E. MILLAR and L. M. TURK, of the Michigan State College. It presents the basic principles of soil science in easily understood terms. It is practical, and broad. Other agricultural fields to which soil science contributes are brought into the discussion, and the general relations of soil to society are recognized.

The development is fairly logical. The first chapter deals with soil development, the second with soil classification, and the third with the physical and chemical properties of soils. Then the authors take up soil reaction,

lime and its uses, soil moisture, soil organisms, and soil organic matter. Following these chapters, cover and green manure crops, farm manures, and the nutrient requirements of plants are considered, and the soil amendments, fertilizers, fertilizer practices, and the maintenance of soil fertility and productivity of the soil. The remainder of the chapters are: Soils and agriculture of arid regions; irrigation; fruit soils; lawn soils; and soil resources. The reviewer considers it a very good survey of soil science at the present time, and recommends it to students and instructors. It is a considerable improvement over many an older text.

The publishers are John Wiley and Sons, Inc., New York, N. Y., who quote the price of the volume at $3.75. With index it contains 462 pages; it is illustrated with 78 figures. A glossary is provided for those who are unfamiliar with the terms used.

Annual Review of Physiology.—The fifth volume of the *Annual Review of Physiology* contains 24 reviews, two of which are in two parts, so that there are really 26. They deal almost exclusively with animal physiology, although such reviews as those by C. V. TAYLOR on the physical aspects of protoplasm, and by J. SCHULTZ on physiological aspects of genetics are certainly of more general interest. In addition to these topics, we note reviews dealing with the physiological and pathological aspects of ultraviolet radiation; developmental physiology; physiology of bone; energy metabolism; the respiratory system; muscle; the digestive system; blood; the lymphatic system; heart; nerve and synaptic transmission; visceral functions of the nervous system; temperature regulation; liver and bile; sense organs, (I) vision; (II) special senses other than vision; metabolic functions of the endocrine system; physiology of mammalian semen; endocrinology of reproduction; physiological psychology, (I) the functional psychoses; (II) physiological correlates of behavior; and biological assay. There is an author index, and a subject index, as usual.

The reviews are prepared by experts in the respective fields, and under the present disturbed world conditions it is remarkable that the authors can do as well as they do. This annual review is serving the field of physiology admirably, and it deserves the support of those who need the summaries, and that includes the physiological and medical fraternity generally.

The price of the *Annual Review of Physiology* is $5.00 per copy. It contains 612 pages, and copies may be ordered from Annual Reviews, Inc., Stanford University P.O., California.